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(54) Title: DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

(57) Abstract

The duplex DNA of chromosomes is replicated in a multicomponent process. A helicase unwinds the DNA, a replicase synthesizes new DNA, and primase repeatedly synthesizes new primed starts on the lagging strand. The present invention is directed to the genes from Gram positive bacterium encoding these proteins, and their characterization. Replicases are highly efficient polymerases. There are several mechanisms by which a replicase can achieve high processivity. The invention determines that the replicase of *Staphylococcus* operates as a 3 component system in which a clamp loader enzyme assembles a sliding clamp protein onto DNA. The sliding clamp then binds the DNA polymerase III holoenzyme making it highly efficient. The invention identifies two DNA polymerase III enzymes in Gram positive bacterium, each of which operate with the clamp and clamp loader, to extend a single primed site around a long (over 5kb) ssDNA template. These replication proteins can be utilized in a variety of assays to screen chemical compound libraries for an antibiotic compound.

DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

This application claims benefit of U.S. Provisional Patent Application

Nos. 60/074,572 and 60/093,727, filed January 27, 1998, and July 22, 1998,

respectively

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FIELD OF THE INVENTION

This invention relates to genes and proteins that replicate the

chromosome of Gram positive bacteria. These proteins can be used in drug discovery

15   to screen large libraries of chemicals for identification of compounds with antibiotic

activity.

BACKGROUND OF THE INVENTION

20   All forms of life must duplicate the genetic material to propagate the

species. The process by which the DNA in a chromosome is duplicated is called

replication. The replication process is performed by numerous proteins that

coordinate their actions to smoothly duplicate the DNA. The main protein actors are

as follows (reviewed in Kornberg, et al., DNA Replication, Second Edition, New

25   York: W.H. Freeman and Company, pp. 165-194 (1992)). A helicase uses the energy

of ATP hydrolysis to unwind the two DNA strands of the double helix. Two copies

of the DNA polymerase use each "daughter" strand as a template to convert them into

two new duplexes. The DNA polymerase acts by polymerizing the four monomer

unit building blocks of DNA (the 4 dNTPs, or deoxynucleoside triphosphates are:

30   dATP, dCTP, dGTP, dTTP). The polymerase rides along one strand of DNA using it

as a template that dictates the sequence in which the monomer blocks are to be

polymerized. Sometimes the DNA polymerase makes a mistake and includes an

incorrect nucleotide (e.g., A instead of G). A proofreading exonuclease examines the polymer as it is made and excises building blocks that have been improperly inserted in the polymer.

Duplex DNA is composed of two strands that are oriented antiparallel

5   to one another, one being oriented 3'-5' and the other 5' to 3'. As the helicase unwinds the duplex, the DNA polymerase moves continuously forward with the helicase on one strand (called the leading strand). However, due to the fact that DNA polymerases can only extend the DNA forward from a 3' terminus, the polymerase on the other strand extends DNA in the opposite direction of DNA unwinding (called the

10   lagging strand). This necessitates a discontinuous ratcheting motion on the lagging strand in which the DNA is made as a series of Okazaki fragments. DNA polymerases cannot initiate DNA synthesis *de novo*, but require a primed site (i.e. a short duplex region). This job is fulfilled by primase, a specialized RNA polymerase, that synthesizes short RNA primers on the lagging strand. The primed sites are

15   extended by DNA polymerase. A single stranded DNA binding protein (SSB) is also needed; it operates on the lagging strand. The function of SSB is to coat single stranded DNA (ssDNA), thereby melting short hairpin duplexes that would otherwise impede DNA synthesis by DNA polymerase.

The replication process is best understood for the Gram negative

20   bacterium, *Escherichia coli*, and its bacteriophages T4 and T7 (reviewed in Kelman, et al., "DNA Polymerase III Holoenzyme: Structure and Function of Chromosomal Replicating Machine," Annu. Rev. Biochem., 64:171-200 (1995); Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev. Biochem., 61:673-719 (1992); McHenry, C.S., "DNA Polymerase III Holoenzyme: Components, Structure, and

25   Mechanism of a True Replicative Complex," J. Bio. Chem., 266:19127-19130 (1991); Young et. al., "Structure and Function of the Bacteriophage T4 DNA Polymerase Holoenzyme," Am. Chem. Soc., 31:8675-8690 (1992)). The eukaryotic systems of yeast (*Saccharomyces cerevisae*) (Morrison et. al., "A Third Essential DNA Polymerase in *S. cerevisiae*," Cell, 62:1143-51 (1990) and humans (Bambara, et al.,

30   "Reconstitution of Mammalian DNA Replication," Prog. Nuc. Acid Res.," 51:93-123 (1995)) have also been characterized in some detail as has herpes virus (Boehmer, et al., "Herpes Simplex Virus DNA Replication," Annu. Rev. Biochem., 66:347-384

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(1997)) and vaccinia virus (McDonald, et. al., "Characterization of a Processive Form of the Vaccinia Virus DNA Polymerase," Virology, 234:168-175 (1997)). The helicase of *E. coli* is encoded by the dnaB gene and is called the DnaB-helicase. In phage T4, the helicase is the product of the gene 41, and, in T7, it is the product of

5   gene 4. Generally, the helicase contacts the DNA polymerase, in *E. coli*. This contact is necessary for the helicase to achieve the catalytic efficiency needed to replicate a chromosome (Kim, et. al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996)). The identity of the helicase that acts at the replication fork in a eukaryotic

10   cellular system is still not firm.

The primase of *E. coli* (product of the dnaG gene), phage T4 (product of gene 61), and T7 (gene 4) require the presence of their cognate helicase for activity. The primase of eukaryotes, called DNA polymerase alpha, looks and behaves differently. DNA polymerase alpha is composed of 4 subunits. The primase

15   activity is associated with the two smaller subunits, and the largest subunit is the DNA polymerase which extends the product of the priming subunits. DNA polymerase alpha does not need a helicase for priming activity.

The chromosomal replicating DNA polymerase of all these systems, prokaryotic and eukaryotic, share the feature that they are processive, meaning they

20   remain continuously associated with the DNA template as they link monomer units (dNTPs) together . This catalytic efficiency can be manifest *in vitro* by their ability to extend a single primer around a circular single stranded DNA (ssDNA) of over 5,000 nucleotide units in length. Chromosomal DNA polymerases will be referred to here as replicases to distinguish them from DNA polymerases that function in other

25   DNA metabolic processes and are far less processive.

There are three types of replicases known thus far that differ in how they achieve processivity, and how their subunits are organized. These will be referred to here as Types I-III. The Type I is exemplified by the phage T5 replicase, which is composed of only one subunit yet is highly processive (Das, et al.,

30   "Mechanism of Primer-template Dependent Conversion of dNTP-dNMP by T7 DNA Polymerase," J. Biol. Chem., 255:7149-7154 (1980)). It is possible that the T5 enzyme achieves processivity by having a cavity within it for binding DNA, and that

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a domain of the protein acts as a lid that opens to accept the DNA, and closes to trap the DNA inside, thereby keeping the polymerase on DNA during polymerization of dNTPs. Type II is exemplified by the replicases of phage T7, herpes simplex virus, and vaccinia virus. In these systems, the replicase is composed of two subunits, the

5   DNA polymerase and an "accessory protein" which is needed for the polymerase to become highly efficient. It is presumed that the DNA polymerase binds the DNA in a groove and that the accessory protein forms a cap over the groove trapping the DNA inside for processive action. Type III is exemplified by the replicases of *E. coli*, phage T4, yeast, and humans in which there are three separate components, a sliding

10   clamp protein, a clamp loader protein complex, and the DNA polymerase. In these systems, the sliding clamp protein is an oligomer in the shape of a ring. The clamp loader is a multiprotein complex which uses ATP to assemble the clamp around DNA. The DNA polymerase then binds the clamp which tethers the polymerase to DNA for high processivity. The replicase of the *E. coli* system contains a fourth component

15   called tau that acts as a glue to hold two polymerases and one clamp loader together into one structure called Pol III*. In this application, any replicase that uses a minimum of three components (i.e. clamp, clamp loader, and DNA polymerase) will be referred to as either a type III enzyme or as a DNA polymerase III-type replicase.

        The *E. coli* replicase is also called DNA polymerase III holoenzyme.

20   The holoenzyme is a single multiprotein particle that contains all the components and therefore is composed of 10 different proteins. This holoenzyme is suborganized into four functional components called: 1) Pol III core (DNA polymerase); 2) gamma complex (clamp loader); 3) beta subunit (sliding clamp); and 4) tau (glue protein). The DNA polymerase III "core" is a tightly associated complex containing one each

25   of the following three subunits: 1) the alpha subunit is the actual DNA polymerase (129 kDa); 2) the epsilon subunit (28 kDa) contains the proofreading 3'-5' exonuclease activity; and 3) the theta subunit has an unknown function. The gamma complex is the clamp loader and contains the following subunits: gamma, delta, delta prime, chi and psi (U.S. Patent No. 5,583,026 to O'Donnell). The beta subunit is a

30   homodimer and forms the ring shaped sliding clamp. These components associate to form the holoenzyme and the entire holoenzyme can be assembled *in vitro* from 10 isolated pure subunits (U.S. Patent No. 5,583,026 to O'Donnell; U.S. Patent No.

5,668,004 to O'Donnell). The tau subunit, encoded by the same gene that encodes gamma (dnaX), acts as a glue to hold two cores together with one gamma complex. This subassembly is called DNA polymerase III star (Pol III*). One beta ring interacts with each core in Pol III* to form DNA polymerase III holoenzyme.

5   During replication, the two cores in the holoenzyme act coordinately to synthesize both strands of DNA in a duplex chromosome. At the replication fork, DNA polymerase III holoenzyme physically interacts with the DnaB helicase through the tau subunit to form a yet larger protein complex termed the "replisome" (Kim, et. al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction

10   Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996); Yuzhakov, et. al., "Replisome Assembly Reveals the Basis for Asymmetric Function in Leading and Lagging Strand Replication," Cell, 86:877-886 (1996)). The primase repeatedly contacts the helicase during replication fork movement to synthesize RNA primers on the lagging strand (Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev.

15   Biochem., 61:673-719 (1992)).

            In the present invention, new genes from Gram positive bacteria (e.g., *S. aureus*) are identified. Although their homology with *E. coli* proteins is often weak, they will be assigned names based on their nearest homology to subunits in the *E. coli* system. The gene of *E. coli* replication proteins are as follows: alpha (dnaE);

20   epsilon (dnaQ); theta (holE); tau (dnaX); gamma (dnaX); delta (holA); delta prime (holB); chi (holC); psi (holD); beta (dnaN); DnaB; helicase (dnaB); and primase (dnaG).

            The dnaX gene encodes both tau and gamma. Tau is the product of the full gene. Gamma is the product of the first 2/3 of the gene; it is truncated by an

25   efficient translational frameshift that results in incorporation of one unique residue followed by a stop codon.

            Although there are many studies of replication mechanisms in eukaryotes, and the Gram negative bacterium, *E. coli* and its bacteriophages, there is very little information about how Gram positive organisms replicate. The

30   evolutionary split between Gram positive bacteria and Gram negative bacteria occurred approximately 1.2 billion years ago. The Gram positive class of bacteria includes some of the worst human pathogens such as *Staphylococcus aureus*,

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Streptococcus pneumoniae, *Streptococcus pyogenes*, *Enterococcus faecalis*, and
Mycobacterium tuberculosis (Youmans, et. al., The Biological and Clinical Basis of
Infectious Disease (1985)).

5   Currently, the best characterized Gram positive organism for DNA
synthesis is *Bacillus subtilis*. Fractionation of *B. subtitis* has identified three DNA
polymerases. Gass, et al., "Further Genetic and Enzymological Characterization of
the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," J. Bio. Chem.,
248:7688-7700 (1973); Ganesan, et. al.; "DNA Replication in a Polymerase I
Deficient Mutant and the Identification of DNA Polymerases II and III in *Bacillus*

10   *subtilis*," Biochem. And Biophy. Res. Commun., 50:155-163 (1973)). These
polymerases are thought to be analogous to the three DNA polymerases of *E. coli*
(DNA polymerases I, II and III). Studies in *B. subtilis* have identified a polymerase
that appears to be involved in chromosome replication and is termed Pol III (Ott, et.
al.; "Cloning and Characterization of the PolC Region of *Bacillus subtilis*," J.

15   Bacteriol., 165:951-957 (1986); Barnes, et. al., "Localization of the Exonuclease and
Polymerase Domains of *Bacillus subtilis* DNA Polymerase III," Gene, 111:43-49
(1992); Barnes, et. al., "The 3'-5' Exonuclease Site of DNA Polymerase III From
Gram-positive Bacteria: Definition of a Novel Motif Structure," Gene" 165:45-50
(1995) or Barnes, et al., "Purification of DNA Polymerase III of Gram-positive

20   Bacteria," Methods in Enzy., 262:35-42 (1995)). The *B. subtilis* Pol III (called PolC)
is larger (about 165 kDa) than the *E. coli* alpha subunit (about 129 kDa) and exhibits
3'-5' exonuclease activity. The PolC gene encoding this Pol III shows weak
homology to the genes encoding *E. coli* alpha and the *E. coli* epsilon subunit. Hence,
this long form of the *B. subtilis* Pol III (herein referred to as Pol III-L) essentially

25   comprises both the alpha and epsilon subunits of the *E. coli* core polymerase. The *S.*
aureus Pol III-L has also been sequenced, expressed in *E. coli* and purified; it contains
polymerase and 3'-5' exonuclease activity (Pacitti, et. al., "Characterization and
Overexpression of the Gene Encoding *Staphylococcus aureus* DNA Polymerase III,"
Gene, 165:51-56 (1995)). Although this Pol III-L is essential to cell growth

30   (Clements, et. al., "Inhibition of *Bacillus subtilis* Deoxyribonucleic Acid Polymerase
III by Phenylhydrazinopyrimidines: Demonstration of a Drug-induced
Deoxyribonucleic Acid-Enzyme Complex," J. Biol. Chem., 250:522-526 (1975);

Cozzarelli, et al., "Mutational Alteraction of *Bacillus subtilis* DNA Polymerase III to Hydroxyphenylazopyrimidine Resistance: Polymerase III is Necessary for DNA Replication," Biochem. And Biophy. Res. Commun., 51:151-157 (1973); Low, et. al., "Mechanism of Inhibition of *Bacillus subtilis* DNA Polymerase III by the

5   Arylhydrazinopyrimidine Antimicrobial Agents," Proc. Natl. Acad. Sci. USA, 71:2973-2977 (1974)), there could still be another DNA polymerase(s) that is essential to the cell, such as occurs in yeast (Morrison, et. al., "A Third Essential DNA Polymerase in *S. cerevisiae*," Cell, 62:1143-1151 (1990)).

Purification of Pol III-L from *B. subtilis* results in only this single

10 protein without associated proteins Barnes, et. al., "Localization of the Exonuclease and Polymerase Domains of *Bacillus subtilis* DNA Polymerase III," Gene, 111:43-49 (1992); Barnes, et. al., "The 3'-5' Exonuclease Site of DNA Polymerase III From Gram-positive Bacteria: Definition of a Novel Motif Structure," Gene" 165:45-50 (1995) or Barnes, et al., "Purification of DNA Polymerase III of Gram-positive

15 Bacteria," Methods in Enzy., 262:35-42 (1995)). Hence, it is possible that Pol III-L is a member of the Type I replicase (like T5) in which it is processive on its own without accessory proteins. *B. subtilis* and *S. aureus* also have a gene encoding a protein that has approximately 30% homology to the beta subunit of *E. coli*; however the protein product has not been purified or characterized (Alonso, et al., "Nucleotide

20 Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 246:680-686 (1995); Alonso, et al., "Nucleotide Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 248:635-636 (1995)). Whether this beta subunit has a

25 function in replication, a ring shape, or functions as a sliding clamp is not known. Even if this beta homolog is involved in replication, it is not known whether it is functional with Pol III-L or another polymerase.

There remains a need to understand the process of DNA replication in Gram positive cells at a molecular level. It is possible that a more detailed

30 understanding of replication proteins will lead to discovery of new antibiotics. Therefore, a deeper understanding of replication proteins of Gram positive bacteria, particularly members of the *Staphylococcus* genus is especially important given the

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emergence of drug resistant strains of these organisms. For example, *Staphylococcus aureus* has successfully mutated to become resistant to all common antibiotics.

The "target" protein(s) of an antibiotic drug is generally involved in a critical cell function, such that blocking its action with a drug causes the pathogenic

5   cell to die or no longer proliferate. Current antibiotics are directed to very few targets. These include membrane synthesis proteins (e.g. vancomycin, penicillin, and its derivatives such as ampicillin, amoxicillin, and cephalosporin), the ribosome machinery (tetracycline, chloramphenicol, azithromycin, and the aminoglycosides: kanamycin, neomycin, gentamicin, streptomycin), RNA polymerase (rifampimycin),

10   and DNA topoisomerases (novobiocin, quinolones, and fluoroquinolones). The DNA replication apparatus is a crucial life process, and, thus, the proteins involved in this process are also good targets for antibiotics.

A powerful approach to discovery of a new drug is to obtain a target protein, characterize it, and develop *in vitro* assays of its cellular function. Large

15   chemical libraries are then screened in the functional assays to identify compounds that inhibit the target protein. These candidate pharmaceuticals are then chemically modified to optimize their potency, breadth of antibiotic spectrum, performance in animal models, non toxicity, and, finally, clinical trials. The screening of large chemical libraries requires a plentiful source of the target protein. An abundant

20   supply of protein generally requires overproduction techniques using the gene encoding the protein. This is especially true for replication proteins as they are present in low abundance in the cell.

Selective and robust assays are needed to screen reliably a large chemical library. The assay should be insensitive to most chemicals in the

25   concentration range normally used in the drug discovery process. These assays should also be selective and not show inhibition by antibiotics known to target proteins in processes outside of replication. The present invention is directed to overcoming these deficiencies in the art.

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SUMMARY OF THE INVENTION

The present invention relates to various isolated DNA molecules from Gram positive bacteria. These include dnaE, dnaX, dnaB, PolC, dnaN, beta, and

5   dnaG DNA molecules for Gram positive bacteria. These DNA molecules can be inserted into an expression system or used to transform host cells. The isolated proteins encoded by these DNA molecules are also disclosed.

The present invention aims to understand the structure and mechanism of the chromosomal replicase of Gram positive bacteria and how it functions with a

10   helicase and primase. This knowledge and the enzymes involved in the replication process can be used for the purpose of screening for potential antibiotic drugs. Further, information about chromosomal replicases may be useful in DNA sequencing, polymerase chain reaction, and other DNA polymerase related techniques.

15   The present invention identifies the type of replicase that Gram positive bacteria employ for chromosome replication. Rather than use a DNA polymerase that attains high efficiency on its own, or with one other subunit, the Gram positive bacteria replicase is the Pol III-type of replicase (class III) that uses a sliding clamp protein. The Gram positive bacteria replicase also uses a clamp loader

20   component that assembles the sliding clamp onto DNA.

The present invention identifies two DNA polymerases (both of Pol III type) in Gram positive bacteria that utilize the sliding clamp and clamp loader. The invention also identifies a gene with homology to the alpha subunit of *E. coli* DNA polymerase III holoenzyme, the chromosomal replicase of *E. coli*. These DNA

25   polymerases can extend a primer around a large circular natural template when the beta clamp has been assembled onto the primed ssDNA by the clamp loader or a primer on a linear DNA where the beta clamp may assemble by itself by sliding over an end.

The present invention shows that the clamp and clamp loader

30   components of Gram negative cells can be exchanged for those of Gram positive cells in that the clamp, once assembled onto DNA, will function with Pol III obtained from either Gram positive and Gram negative sources. This result implies that important

contacts between the polymerase and clamp have been conserved during evolution. Therefore, these "mixed systems" may provide assays for an inhibitor of this conserved interaction. Such an inhibitor may be expected to shut down replication, and since the interaction is apparently conserved across the evolutionary spectrum

5   from Gram positive and Gram negative cells, the inhibitor may exhibit a broad spectrum of antibiotic activity. Further, these "mixed" systems are composed of all overexpressed and purified proteins (8 total; 1 from *S. aureus* and 7 from *E. coli*) making possible large quantities of protein needed for high throughput screening of hundreds of thousands of chemicals.

10   The present invention demonstrates that Gram positive bacteria contain a beta subunit that behaves as a sliding clamp that encircles DNA. A dnaX gene sequence encoding a protein homolog of the gamma/tau subunit of the clamp loader (gamma complex) *E. coli* DNA polymerase III holoenzyme is also identified. The presence of this gene confirms the presence of a clamp loading apparatus in Gram

15   positive bacteria that will assemble beta clamps onto DNA for the DNA polymerases.

             A new gene sequence encoding a DNA polymerase homologous to the alpha subunit of DNA polymerase III holoenzyme of *E. coli* (referred to herein as dnaE homolog) is also identified.

             Also identified is a new gene sequence encoding a homolog of the

20   replicative dnaB helicase of *E. coli*.

             This application also outlines methods and assays for use of these replication proteins in drug screening processes.

                        BRIEF DESCRIPTION OF THE DRAWINGS

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             Figure 1 shows the construction of the *S. aureus* Pol III-L expression vector. The gene encoding Pol III-L was cloned into a pET11 expression vector in a three step cloning scheme as illustrated.

             Figures 2A-C describe the expression and purification of *S. aureus* Pol

30   III-L. Figure 2A compares *E. coli* cells that contain the pET11PolC expression vector that are either induced or uninduced for protein expression. The gel is stained with Coomassie Blue. The induced band corresponds to the expected molecular weight of

the *S. aureus* Pol III-L, and is indicated to the right of the gel. Figure 2B shows the
results of the MonoQ chromatography of a lysate of *E. coli* (pET11PolC-L) induced
for Pol III-L. The fractions were analyzed in a Coomassie Blue stained gel (top) and
for DNA synthesis (bottom). Fractions containing Pol III-L are indicated. In

5   Figure 2C, fractions containing Pol III-L from the MonoQ column were pooled and
chromatographed on a phosphocellulose column. This shows an analysis of the
column fractions from the phosphocellulose column in a Coomassie Blue stained
polyacrylamide gel. The position of Pol III-L is indicated to the right.

Figure 3 shows the *S. aureus* beta expression vector. The dnaN gene

10   was amplified from *S. aureus* genomic DNA and cloned into the pET16 expression
vector.

Figures 4A-C describe the expression and purification of *S. aureus*
beta. Figure 4A compares *E. coli* cells that contain the pET16beta expression vector
that are either induced or uninduced for protein expression. The gel is stained with

15   Coomassie Blue. The induced band corresponds to the expected molecular weight of
the *S. aureus* beta, and is indicated to the right of the gel. The migration position of
size standards are indicated to the left. Figure 4B shows the results of MonoQ
chromatography of an *E. coli* (pET16beta) lysate induced for beta. The fractions were
analyzed in a Coomassie Blue stained gel, and fractions containing beta are indicated.

20   In Figure 4C, fractions containing beta from the MonoQ column were pooled and
chromatographed on a phosphocellulose column. This shows an analysis of the
column fractions from the phosphocellulose column in a Coomassie Blue stained
polyacrylamide gel. The position of beta is indicated to the right.

Figures 5A-B demonstrate that the *S. aureus* beta stimulates *S. aureus*

25   Pol III-L and *E. coli* Pol III core on linear DNA, but not circular DNA. In Figure 5A,
the indicated proteins were added to replication reactions containing polydA-oligodT
as described in the Examples *supra*. Amounts of proteins added, when present, were:
lanes 1,2: *S. aureus* Pol III-L, 7.5 ng; *S. aureus* b, 6.2 ug; Lanes 3,4: *E. coli* Pol III
core, 45 ng; *S. aureus* b, 9.3 ug; Lanes 5,6: *E. coli* Pol III core, 45 ng; *E. coli* b, 5μg.

30   Total DNA synthesis was: Lanes 1-6: 4.4, 30.3, 5.1, 35.5, 0.97, 28.1 pmol,
respectively. In Figure 5B, Lanes 1-3, the indicated proteins were added to
replication reactions containing circular singly primed M13mp18 ssDNA as described

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in the Example *supra. S. aureus* b, 0.8 ug; *S. aureus* Pol III-L, 300 ng (purified

through MonoQ); *E. coli* gamma complex, 1.7 μg. Results in the *E. coli* system are

shown in Lanes 4-6. Total DNA synthesis was: Lanes 1-6: 0.6, 0.36, 0.99, 2.7, 3.5,

280 pmol, respectively.

5   Figure 6 shows that *S. aureus* Pol III-L functions with *E. coli* beta and

gamma complex on circular primed DNA. It also shows that *S. aureus* beta does not

convert Pol III-L with sufficient processivity to extend the primer all the way around a

circular DNA. Replication reactions were performed on the circular singly primed

M13mp18 ssDNA. Proteins added to the assay were as indicated in this figure. The

10   amount of each protein, when present, was: *S. aureus* beta, 800 ng; *S. aureus* Pol III-

L, 1500 ng (MonoQ fraction 64); *E. coli* Pol III core, 450 ng; *E. coli* beta, 100 ng; *E.

coli* gamma complex, 1720 ng. Total DNA synthesis in each assay is indicated at the

bottom of the figure.

          Figures 7A-B show that *S. aureus* contains four distinct DNA

15   polymerases. Four different DNA polymerases were partially purified from *S. aureus*

cells. *S. aureus* cell lysate was separated from DNA and, then, chromatographed on a

MonoQ column. Fractions were analyzed for DNA polymerase activity. Three peaks

of activity were observed. The second peak was the largest and was expected to be a

mixture of two DNA polymerases based on early studies in *B. subtilis*.

20   Chromatography of the second peak on phosphocellulose (Figure 7B) resolved two

DNA polymerases from one another.

          Figures 8A-B show that *S. aureus* has two DNA Pol III's. The four

DNA polymerases partially purified from *S. aureus* extract, designated peaks I-IV in

Figure 7, were assayed on circular singly primed M13mp18 ssDNA coated with *E.*

25   *coli* SSB either in the presence or absence of *E. coli* beta (50ng) and gamma complex

(50 ng). Each reaction contained 2 μl of the partially pure polymerase (Peak 1 was

Mono Q fraction 24 (1.4 ug), Peak 2 was phosphocellulose fraction 26 (0.016 mg/ml),

Peak 3 was phosphocellulose fraction 46 (0.18 mg/ml), and Peak 4 was MonoQ

fraction 50 (1 ug). Figure 8A shows the product analysis in an agarose gel.

30   Figure 8B shows the extent of DNA synthesis in each assay.

          Figure 9 compares the homology between the polypeptide encoded by

dnaE of *S. aureus* and other organisms. An alignment is shown for the amino acid

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sequence of the *S. aureus* dnaE product with the dnaE products (alpha subunits) of *E. coli* and *Salmonella typhimurium*.

Figure 10 compares the homology between the N-terminal regions of the gamma/tau polypeptides of *S. aureus*, *B. subtilis*, and *E. coli*. The conserved ATP site and the cystines forming the zinc finger are indicated above the sequence. The organisms used in the alignment were: *E. coli* (GenBank); and *B. subtilis*.

Figure 11 compares the homology between the DnaB polypeptide of *S. aureus* and other organisms. The organisms used in the alignment were: *E. coli* (GenBank); *B. subtilis*; *Sal.Typ.*, (*Salmonella typhimurium*).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to various isolated DNA molecules from Gram positive bacteria. These include dnaE, dnaX, dnaB, PolC, dnaN, and dnaG DNA molecules from Gram positive bacteria. These DNA molecules can be inserted into an expression system or used to transform host cells. The isolated proteins encoded by these DNA molecules are also disclosed.

These DNA molecules and proteins can be derived from any Gram positive bacteria, including *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Mycobacterium*. It is particularly directed to such DNA molecules and proteins derived from *Staphylococcus* bacteria, particularly *Staphylococcus aureus*.

One aspect of the present invention relates to a newly discovered Pol III gene of *S. aureus* cells (herein identified as dnaE) that is homologous to *E. coli* alpha (product of dnaE gene). The partial DNA sequence of the *S. aureus* dnaE gene is as follows (SEQ. ID. No. 1):

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GATATAGATA TGGACTGGGA AGATACACGC CGAGAAAAGG TCATTCAGTA CGTCCAAGAA      60

AAATATGGCG AGCTACATGT ATCTGGAATT GTGACTTTCG GTCATCTGCT TGCAAAAGCG     120

GTTGCTAAAG ATGTTGGACG AATTATGGGG TTTGATGAAG TTACATTAAA TGAAATTTCA     180

AGTTTAATCC CACATAAATT AGGAATTACA CTTGATGAAG CATATCAAAT TGACGATTTT     240

AAAAAGTTTG TACATCGAAA CCATCGACAT CAACGCTGGT TCAGTATTTG TAAAAAGTTA     300

GAAGGTTTAC CAAGACATAC ATCTACACAT GCGGCAGGAA TTATTATTAA TGACCATCCA     360
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      TTATATGAAT ATGCCCCTTT AACGAAAGGG GATACAGGAT TATTAACGCA ATGGACAATG    420

      ACTGAAGCCG AACGTATTGG TTTATTAAAA ATAGATTTTC TAGGGTTACG AAATTTATCA    480

      ATTATCCATC AAATTTTGAC TCGAGTCGAA AAAGATTTAG GTTTTAATAT TGATATTGAA    540

      AAAATTCCAT TTGATGATCA AAAAGTGTTT GAATTGTTGT CGCAAGGAGA TACGACTGGC    600

      ATATTTCAAT TAGAGTCTGA CGGTGTTAGA AGTGTATTAA AAAAATTAAA GCCGGAACAC    660

      TTTGAGGATA TTGTTGCTGT AACTTCTTTG TATAGACCAG GTCCAATGGA AGAAATTCCA    720

      ACTTACATTA CAAGAAGACA TGATCCAAGC AAAGTTCAAT ATTTACATCC CCATTTAGAA    780

      CCTATATTAA AAAATACTTA CGGTGTTATT ATTTATCAAG AACAAATTAT GCAAATAGCG    840

      AGCACTTTTG CAAACTTCAG TTATGGTGAA GCGGATATTT TAAGAAGAGC AATGAGTAAA    900

      AAAAATAGAG CTGTTCTTGA AAGAGACGCT CAACATTTTA TAGAAGGTAC AAAGCAAAAT    960

      GGTTATCACG AAGACTTAGT AAGTAAGCAG ATATTTGATT TGATTCTGAA ATTTGCTGAT   1020

      GATGGATTTC CTAGAGCACA TGCTGTCAGC TATTCTAAAA TTGCATACAT TATGAGCTTT   1080

      TTAAAAGTCC ATTATCCAAA TTATTTTTAC GCAAATATTT TAAGTAATGT TATTGGAAGT   1140

      GAGAAGAAAA CTGCTCAAAT GATAGAAGAA GCAAAAAAAC AAGGTATCAC TATATTGCCA   1200

      CCGAACATTA ACGAAAGTCA TTGGTTTTAT AAACCTTCCC AAGAAGGCAT TTATTTATCA   1260

      ATTGGTACAA TTAAAGGTGT AGGTTATCAA AGTGTGAAAG TGATTGTTGA AGAACGTTTT   1320

      CAGAACGGCA AATTTAAAGA TTTCTTTGAT TCTGCTAGAC GTATACCGAA GAGAGTCAAA   1380

      ACGAGAAAGT TACTTGAAGC ATTGATTTTA GTGGGAGCGT TTGATGCTTT TGGTAAAACA   1440

      CGTTCAACGT TGTTGCAAGC TATTGATCAA GTGTTGGATG GTGATTTAAA CATTGAACAA   1500

      GATGGTTTTT TATTTGATAT TTTAACGCCA AAACAGATGT ATGAAGATAA AGAAGAATTG   1560

      CCTGATGCAC TTATTAGTCA GTATGAAAAA GAATATTTAG GATTTTATGT TTCGCAACAC   1620

      CCAGTAGATA AGAAGTTTGT TGCCAAACAA TATTTAACGA TATTTTCTTG CGAAAACGTT   1680

      GCTAAAGATG TTCGACGAAT TATGGGGTTT GATGAAGTTA AACAAA               1726
```

The *S. aureus* dnaE encoded protein has a partial amino acid sequence as follows (SEQ. ID. No. 2):

```
      Asp Ile Asp Met Asp Trp Glu Asp Thr Arg Arg Glu Lys Val Ile Gln
      1             5                   10                  15

      Tyr Val Gln Glu Lys Tyr Gly Glu Leu His Val Ser Gly Ile Val Thr
                    20                  25                  30
```

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```
         Phe Gly His Leu Leu Ala Lys Ala Val Ala Lys Asp Val Gly Arg Ile
             35                  40              45

 5       Met Gly Phe Asp Glu Val Thr Leu Asn Glu Ile Ser Ser Leu Ile Pro
             50                  55              60

         His Lys Leu Gly Ile Thr Leu Asp Glu Ala Tyr Gln Ile Asp Asp Phe
         65                  70              75              80

10       Lys Lys Phe Val His Arg Asn His Arg His Gln Arg Trp Phe Ser Ile
                     85              90                  95

         Cys Lys Lys Leu Glu Gly Leu Pro Arg His Thr Ser Thr His Ala Ala
                     100             105             110
15
         Gly Ile Ile Ile Asn Asp His Pro Leu Tyr Glu Tyr Ala Pro Leu Thr
                     115             120             125

20       Lys Gly Asp Thr Gly Leu Leu Thr Gln Trp Thr Met Thr Glu Ala Glu
             130                 135             140

         Arg Ile Gly Leu Leu Lys Ile Asp Phe Leu Gly Leu Arg Asn Leu Ser
         145                 150             155             160

25       Ile Ile His Gln Ile Leu Thr Arg Val Glu Lys Asp Leu Gly Phe Asn
                         165             170             175

         Ile Asp Ile Glu Lys Ile Pro Phe Asp Asp Gln Lys Val Phe Glu Leu
                     180             185             190
30
         Leu Ser Gln Gly Asp Thr Thr Gly Ile Phe Gln Leu Glu Ser Asp Gly
                 195             200             205

35       Val Arg Ser Val Leu Lys Lys Leu Lys Pro Glu His Phe Glu Asp Ile
             210                 215             220

         Val Ala Val Thr Ser Leu Tyr Arg Pro Gly Pro Met Glu Glu Ile Pro
         225                 230             235             240

40       Thr Tyr Ile Thr Arg Arg His Asp Pro Ser Lys Val Gln Tyr Leu His
                     245             250             255

         Pro His Leu Glu Pro Ile Leu Lys Asn Thr Tyr Gly Val Ile Ile Tyr
                     260             265             270
45
         Gln Glu Gln Ile Met Gln Ile Ala Ser Thr Phe Ala Asn Phe Ser Tyr
                     275             280             285

50       Gly Glu Ala Asp Ile Leu Arg Arg Ala Met Ser Lys Lys Asn Arg Ala
             290                 295             300

         Val Leu Glu Arg Asp Ala Gln His Phe Ile Glu Gly Thr Lys Gln Asn
         305                 310             315             320
```

```
Gly Tyr His Glu Asp Ile Ser Lys Gln Ile Phe Asp Leu Ile Leu Lys
                325             330             335

Phe Ala Asp Gly Phe Pro Arg Ala His Ala Val Ser Tyr Ser Lys Ile
            340             345             350

Ala Tyr Ile Met Ser Phe Leu Lys Val His Tyr Pro Asn Tyr Phe Tyr
        355             360             365

Ala Asn Ile Leu Ser Asn Val Ile Gly Ser Glu Lys Lys Thr Ala Gln
        370             375             380

Met Ile Glu Glu Ala Lys Lys Gln Gly Ile Thr Ile Leu Pro Pro Asn
385             390             395             400

Ile Asn Glu Ser His Trp Phe Tyr Lys Pro Ser Gln Glu Gly Ile Tyr
            405             410             415

Leu Ser Ile Gly Thr Ile Lys Gly Val Gly Tyr Gln Ser Val Lys Val
            420             425             430

Ile Val Glu Glu Arg Phe Gln Asn Gly Lys Phe Lys Asp Phe Phe Asp
        435             440             445

Ser Ala Arg Arg Ile Pro Lys Arg Val Lys Thr Arg Lys Leu Leu Glu
    450             455             460

Ala Leu Ile Leu Val Gly Ala Phe Asp Ala Phe Gly Lys Thr Arg Ser
465             470             475             480

Thr Leu Leu Gln Ala Ile Asp Gln Val Leu Asp Gly Asp Leu Asn Ile
            485             490             495

Glu Gln Asp Gly Phe Leu Phe Asp Ile Leu Thr Pro Lys Gln Met Tyr
            500             505             510

Glu Asp Lys Glu Glu Leu Pro Asp Ala Leu Ile Ser Gln Tyr Glu Lys
        515             520             525

Glu Tyr Leu Gly Phe Tyr Val Ser Gln His Pro Val Asp Lys Lys Phe
    530             535             540

Val Ala Lys Gln Tyr Leu Thr Ile Phe Ser Cys Glu Asn Val Ala Lys
545             550             555             560

Asp Val Arg Arg Ile Met Gly Phe Asp Glu Val Lys Gln
            565             570
```

The present invention also relates to the *S. aureus* dnaX gene. This *S. aureus* dnaX gene has a partial nucleotide sequence as follows (SEQ. ID. No. 3):

```
TTGAATTATC AAGCCTTATA TCGTATGTAC AGACCCCAAA GTTTCGAGGA TGTCGTCGGA      60

CAAGAACATG TCACGAAGAC ATTGCGCAAT GCGATTTCGA AAGAAAAACA GTCGCATGCA     120

TATATTTTTA GTGGTCCGAG AGGTACGGGG AAAACGAGTA TTGCCAAAGT GTTTGCTAAA     180

GCAATCAACT GTTTAAATAG CACTGATGGA GAACCTTGTA ATGAATGTCA TATTTGTAAA     240

GGCATTACGC AGGGGACTAA TTCAGATGTG ATAGAAATTG ATGCTGCTAG TAATAATGGC     300

GTTGATGAAA TAAGAAATAT TAGAGACAAA GTTAAATATG CACCAAGTGA ATCGAAATAT     360

AAAGTTTATA TTATAGATGA GGTGCACATG CTAACAACAG GTGCTTTTAA TGCCCTTTTA     420

AAGACGTTAG AAGAACCTCC AGCACACGCT ATTTTTATAT TGGCAACGAC AGAACCACAT     480

AAAATCCCTC CAACAATCAT TTCTAGGGCA CAACGTTTTG ATTTTAAAGC AATTAGCCTA     540

GATCAAATTG TTGAACGTTT AAAATTGTA GCAGATGCAC AACAAATTGA ATGTGAAGAT     600

GAAGCCTTGG CATTTATCGC TAAAGCGTCT GAAGGGGGTA TGCGTGATGC ATTAAGTATT     660

ATGGATCAGG CTATTGCTTT CGGCGATGGC ACATTGACAT TACAAGATGC CCTAAATGTT     720

ACGGGTAGCG TTCATGATGA AGCGTTGGAT CACTTGTTTG ATGATATTGT ACAAGGTGAC     780

GTACAAGCAT CTTTTAAAAA ATACCATCAG TTTATAACAG AAGGTAAAGA AGTGAATCGC     840

CTAATAAATG ATATGATTTA TTTTGTCAGA GATACGATTA TGAATAAAAC ATCTGAGAAA     900

GATACTGAGT ATCGAGCACT GATGAACTTA GAATTAGATA TGTTATATCA AATGATTGAT    960

CTTATTAATG ATACATTAGT GTCGATTCGT TTTAGTGTGA ATCAAAACGT TCATTTTGAA   1020

GTATTGTTAG TAAAATTAGC TGAGCAGATT AAGGGTCAAC CACAAGTGAT TGCGAATGTA   1080

GCTGAACCAG CACAAATTGC TTCATCGCCA AACACAGATG TATTGTTGCA ACGTATGGAA   1140

CAGTTAGAGC AAGAACTAAA AACACTAAAA GCACAAGGAG TGAGTGTTGC TCCTACTCAA   1200

AAATCTTCGA AAAAGCCTGC GAGAGGTATA CAAAAATCTA AAAATGCATT TTCAATGCAA   1260

CAAATTGCAA AAGTGCTAGA TAAAGCGAAT AAGGCAGATA TCAAATTGTT GAAAGATCAT   1320

TGGCAAGAAG TGATTGACCA TGCCCAAAAC AATGATAAAA AATCACTCGT TAGTTTATTG   1380

CAAAATTCGG AACCTGTGGC GGCAAGTGAA GATCACGTCC TTGTGAAATT TGAGGAAGAG   1440

ATCCATTGTG AAATCGTCAA TAAAGACGAC GAGAAACGTA GTAGTATAGA AAGTGTTGTA   1500

TGTAATATCG TTAATAAAAA CGTTAAAGTT GTTGGTGTAC CATCAGATCA ATGGCAAAGA   1560

GTTCGAACGG AGTATTTACA AAATCGTAAA AACGAAGGCG ATGATATGCC AAAGCAACAA   1620

GCACAACAAA CAGATATTGC TCAAAAAGCA AAAGATCTTT TCGGTGAAGA AACTGTACAT   1680

GTGATAGATG AAGAGTGA                                                  1698
```

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The *S. aureus* dnaX protein (i.e. the gamma subunit/tau subunit) has a
partial amino acid sequence as follows (SEQ. ID. No. 4):

```
5     Leu Asn Tyr Gln Ala Leu Tyr Arg Met Tyr Arg Pro Gln Ser Phe Glu
      1               5               10              15

      Asp Val Val Gly Gln Glu His Val Thr Lys Thr Leu Arg Asn Ala Ile
                      20              25              30

10    Ser Lys Glu Lys Gln Ser His Ala Tyr Ile Phe Ser Gly Pro Arg Gly
                  35              40              45

      Thr Gly Lys Thr Ser Ile Ala Lys Val Phe Ala Lys Ala Ile Asn Cys
          50              55              60

15    Leu Asn Ser Thr Asp Gly Glu Pro Cys Asn Glu Cys His Ile Cys Lys
      65              70              75              80

      Gly Ile Thr Gln Gly Thr Asn Ser Asp Val Ile Glu Ile Asp Ala Ala
20                85              90              95

      Ser Asn Asn Gly Val Asp Glu Ile Arg Asn Ile Arg Asp Lys Val Lys
                  100             105             110

25    Tyr Ala Pro Ser Glu Ser Lys Tyr Lys Val Tyr Ile Ile Asp Glu Val
              115             120             125

      His Met Leu Thr Thr Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu
          130             135             140

30    Glu Pro Pro Ala His Ala Ile Phe Ile Leu Ala Thr Thr Glu Pro His
      145             150             155             160

      Lys Ile Pro Pro Thr Ile Ile Ser Arg Ala Gln Arg Phe Asp Phe Lys
35                    165             170             175

      Ala Ile Ser Leu Asp Gln Ile Val Glu Arg Leu Lys Phe Val Ala Asp
                  180             185             190

40    Ala Gln Gln Ile Glu Cys Glu Asp Glu Ala Leu Ala Phe Ile Ala Lys
                  195             200             205

      Ala Ser Glu Gly Gly Met Arg Asp Ala Leu Ser Ile Met Asp Gln Ala
          210             215             220

45    Ile Ala Phe Gly Asp Gly Thr Leu Thr Leu Gln Asp Ala Leu Asn Val
      225             230             235             240

      Thr Gly Ser Val His Asp Glu Ala Leu Asp His Leu Phe Asp Asp Ile
50                245             250             255
```

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```
      Val Gln Gly Asp Val Gln Ala Ser Phe Lys Lys Tyr His Gln Phe Ile
              260             265             270

 5    Thr Glu Gly Lys Glu Val Asn Arg Leu Ile Asn Asp Met Ile Tyr Phe
              275             280             285

      Val Arg Asp Thr Ile Met Asn Lys Thr Ser Glu Lys Asp Thr Glu Tyr
          290             295             300

10    Arg Ala Leu Met Asn Leu Glu Leu Asp Met Leu Tyr Gln Met Ile Asp
      305             310             315             320

      Leu Ile Asn Asp Thr Leu Val Ser Ile Arg Phe Ser Val Asn Gln Asn
                  325             330             335
15
      Val His Phe Glu Val Leu Leu Val Lys Leu Ala Glu Gln Ile Lys Gly
              340                 345             350

20    Gln Pro Gln Val Ile Ala Asn Val Ala Glu Pro Ala Gln Ile Ala Ser
              355             360             365

      Ser Pro Asn Thr Asp Val Leu Leu Gln Arg Met Glu Gln Leu Glu Gln
          370             375             380

25    Glu Leu Lys Thr Leu Lys Ala Gln Gly Val Ser Val Ala Pro Thr Gln
      385             390             395             400

      Lys Ser Ser Lys Lys Pro Ala Arg Gly Ile Gln Lys Ser Lys Asn Ala
                  405             410             415
30
      Phe Ser Met Gln Gln Ile Ala Lys Val Leu Asp Lys Ala Asn Lys Ala
              420             425             430

35    Asp Ile Lys Leu Leu Lys Asp His Trp Gln Glu Val Ile Asp His Ala
              435             440             445

      Gln Asn Asn Asp Lys Lys Ser Leu Val Ser Leu Leu Gln Asn Ser Glu
          450             455             460

40    Pro Val Ala Ala Ser Glu Asp His Val Leu Val Lys Phe Glu Glu Glu
      465             470             475             480

      Ile His Cys Glu Ile Val Asn Lys Asp Asp Glu Lys Arg Ser Ser Ile
                  485             490             495
45
      Glu Ser Val Val Cys Asn Ile Val Asn Lys Asn Val Lys Val Val Gly
              500             505             510

50    Val Pro Ser Asp Gln Trp Gln Arg Val Arg Thr Glu Tyr Leu Gln Asn
              515             520             525

      Arg Lys Asn Glu Gly Asp Asp Met Pro Lys Gln Gln Ala Gln Gln Thr
              530             535             540
```

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```
    Asp Ile Ala Gln Lys Ala Lys Asp Leu Phe Gly Glu Glu Thr Val His
    545             550             555             560
```

```
    Val Ile Asp Glu Glu Glx
                565
```

This invention also relates to the partial nucleotide sequence of the *S. aureus* dnaB gene as follows (SEQ. ID. No. 5):

```
    ATGGATAGAA TGTATGAGCA AAATCAAATG CCGCATAACA ATGAAGCTGA ACAGTCTGTC     60

    TTAGGTTCAA TTATTATAGA TCCAGAATTG ATTAATACTA CTCAGGAAGT TTTGCTTCCT    120

    GAGTCGTTTT ATAGGGGTGC CCATCAACAT ATTTTCCGTG CAATGATGCA CTTAAATGAA    180

    GATAATAAAG AAATTGATGT TGTAACATTG ATGGATCAAT TATCGACGGA AGGTACGTTG    240

    AATGAAGCGG GTGGCCCGCA ATATCTTGCA GAGTTATCTA CAAATGTACC AACGACGCGA    300

    AATGTTCAGT ATTATACTGA TATCGTTTCT AAGCATGCAT TAAAACGTAG ATTGATTCAA    360

    ACTGCAGATA GTATTGCCAA TGATGGATAT AATGATGAAC TTGAACTAGA TGCGATTTTA    420

    AGTGATGCAG AACGTCGAAT TTTAGAGCTA TCATCTTCTC GTGAAAGCGA TGGCTTTAAA    480

    GACATTCGAG ACGTCTTAGG ACAAGTGTAT GAAACAGCTG AAGAGCTTGA TCAAAATAGT    540

    GGTCAAACAC CAGGTATACC TACAGGATAT CGAGATTTAG ACCAAATGAC AGCAGGGTTC    600

    AACCGAAATG ATTTAATTAT CCTTGCAGCG CGTCCATCTG TAGGTAAGAC TGCGTTCGCA    660

    CTTAATATTG CACAAAAAGT TGCAACGCAT GAAGATATGT ATACAGTTAA AAGCAACAGG    720

    AAGTTTCTGA AATCTCTCGT ACATTAAAAG CATTAGCCCG TGAATTAAAA TGTCCAGTTA    780

    TCGCATTAAG TCAGTTATCT CGTGGTGTTG AACAACGACA AGATAAACGT CCAATGATGA    840

    GTGATATTCG TGAATCTGGT TCGATTGAGC AAGATGCCGA TATCGTTGCA TTCTTATACC    900

    GTGATGATTA CTATAACCGT GGCGGCGATG AAGATGATGA CGATGATGGT GGTTTCGAGC    960

    CACAAACGAA TGATGAAAAC GGTGAAATTG AAATTATCAT TGTTAAGCAA CGTAACGGTC   1020

    CAACAGGCAC AGTTAAGTTA CATTTTATGA AACAATATAA TAAATTTTAG AGCTATCATC   1080

    TTTTCGTGAA AGCGATGGCT TTAAAGACAT TCGAGACGTC TTAGGACAAG TGTATGAAAC   1140

    AGCTGAAGAG CTTGATCAAA ATAGTGGTCA AACACCAGGT ATACCTACAG GATATCGAGA   1200

    TTTAGACCAA ATGACAGCAG GGTTCAACCG AAATGATTTA ATTATCCTTG CAGCGCGTCC   1260

    ATCTGTAGGT AAGACTGCGT TCGCACTTAA TATTGCACAA AAAGTTGCAA CGCATCCGCA   1320

    CTTAATATTG CCAATAAGTT GGAACGCATG AAGATATATC TAGCAGTTGG TATTTTCTCA   1380

    CTAGAGATGG GTGCTGATCA GTTAACCACA CGTATGATTT GTAGTTCTGG TAATGTTGAC   1440
```

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```
        TCAAACCGCT  TAAGAACCGG  TACTATGACT  GAGGAAGATT  GGAGTCGTTT  TACTATAGCG      1500

        GTTGGTAAAT  TATCACGTAC  GAAGATTTTT  ATTGATGATA  CACCGGGTAT  TCGAATTAAT      1560

        GATTTACGTT  CTAAATGTCG  TCGATTAAAG  CAAGAACATG  GCTTAGACAT  GATTGTGATT      1620

        GACTACTTAC  AGTTGATTCA  AGGTAGTGGT  TCACGTGCGT  CCGATAACAG  ACAACAGGAA      1680

        GTTTCTGAAA  TCTCTCGTAC  ATTAAAAGCA  TTAGCCCGTG  AATTAAAATG  TCCAGTTATC      1740

        GCATTAAGTC  AGTTATCTCG  TGGTGTTGAA  CAACGACAAG  ATAAACGTCC  AATGATGAGT      1800

        GATATTCGTG  AATCTGGTTC  GATTGAGCAA  GATGCCGATA  TCGTTGCATT  CTTATACCGT      1860

        GATGATTACT  ATAACCGTGG  CGGCGATGAA  GATGATGACG  ATGATGGTGG  TTTCGAGCCC      1920

        CAAACGAATG  ATGAAACGG  TGAAATTGAA  ATTATCATTG  CTAAGCAACG  TTACGGTCCA      1980

        ACAGGCACAG  TTAAGTTACT  TTTTATGAAA  CAATATGGTA  AATTTACCGA  TATC            2034
```

The amino acid sequence of *S. aureus* DnaB encoded by the dnaB gene is as follows (SEQ. ID. No. 6):

```
        Met Asp Arg Met Tyr Glu Gln Asn Gln Met Pro His Asn Asn Glu Ala
        1               5                   10                  15

        Glu Gln Ser Val Leu Gly Ser Ile Ile Ile Asp Pro Glu Leu Ile Asn
                        20                  25                  30

        Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His
                    35                  40                  45

        Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu
                50                  55                  60

        Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu
        65                  70                  75                  80

        Asn Glu Ala Gly Gly Pro Gln Tyr Leu Ala Glu Leu Ser Thr Asn Val
                        85                  90                  95

        Pro Thr Thr Arg Asn Val Gln Tyr Tyr Thr Asp Ile Val Ser Lys His
                    100                 105                 110

        Ala Leu Lys Arg Arg Leu Ile Gln Thr Ala Asp Ser Ile Ala Asn Asp
                    115                 120                 125

        Gly Tyr Asn Asp Glu Leu Glu Leu Asp Ala Ile Leu Ser Asp Ala Glu
                130                 135                 140

        Arg Arg Ile Leu Glu Leu Ser Ser Ser Arg Glu Ser Asp Gly Phe Lys
        145                 150                 155                 160
```

```
      Asp Ile Arg Asp Val Leu Gly Gln Val Tyr Glu Thr Ala Glu Glu Leu
              165             170             175

      Asp Gln Asn Ser Gly Gln Thr Pro Gly Ile Pro Thr Gly Tyr Arg Asp
 5            180             185             190

      Leu Asp Gln Met Thr Ala Gly Phe Asn Arg Asn Asp Leu Ile Ile Leu
              195             200             205

10    Ala Ala Arg Pro Ser Val Gly Lys Thr Ala Phe Ala Leu Asn Ile Ala
          210             215             220

      Gln Lys Leu Glu Arg Met Lys Ile Tyr Leu Ala Val Gly Ile Phe Ser
      225             230             235             240
15
      Leu Glu Met Gly Ala Asp Gln Leu Thr Thr Arg Met Ile Cys Ser Ser
              245             250             255

20    Gly Asn Val Asp Ser Asn Arg Leu Arg Thr Gly Thr Met Thr Glu Glu
              260             265             270

      Asp Trp Ser Arg Phe Thr Ile Ala Val Gly Lys Leu Ser Arg Thr Lys
              275             280             285

25    Ile Phe Ile Asp Asp Thr Pro Gly Ile Arg Ile Asn Asp Leu Arg Ser
          290             295             300

      Lys Cys Arg Arg Leu Lys Gln Glu His Gly Leu Asp Met Ile Val Ile
      305             310             315             320
30
      Asp Tyr Leu Gln Leu Ile Gln Gly Ser Gly Ser Arg Ala Ser Asp Asn
              325             330             335

      Arg Gln Gln Glu Val Ser Glu Ile Ser Arg Thr Leu Lys Ala Leu Ala
35            340             345             350

      Arg Glu Leu Lys Cys Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly
              355             360             365

40    Val Glu Gln Arg Gln Asp Lys Arg Pro Met Met Ser Asp Ile Arg Glu
          370             375             380

      Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg
      385             390             395             400
45
      Asp Asp Tyr Tyr Asn Arg Gly Gly Asp Glu Asp Asp Asp Asp Gly
              405             410             415

50    Gly Phe Glu Pro Gln Thr Asn Asp Glu Asn Gly Glu Ile Glu Ile Ile
              420             425             430

      Ile Ala Lys Gln Arg Tyr Gly Pro Gly Thr Val Lys Leu Leu Phe Met
              435             440             445
```

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```
Lys Gln Tyr Gly Lys Phe Thr Asp Ile
    450                   455
```

The present invention also uses the gene sequence of *S. aureus* PolC
(encoding Pol III-L). The nucleotide sequence is as follows (SEQ. ID. No. 7):

```
ATGACAGAGC AACAAAAATT TAAAGTGCTT GCTGATCAAA TTAAAATTTC AAATCAATTA     60

GATGCTGAAA TTTTAAATTC AGGTGAACTG ACACGTATAG ATGTTTCTAA CAAAAACAGA    120

ACATGGGAAT TCATATTAC ATTACCACAA TTCTTAGCTC ATGAAGATTA TTTATTATTT    180

ATAAATGCAA TAGAGCAAGA GTTTAAAGAT ATCGCCAACG TTACATGTCG TTTTACGGTA    240

ACAAATGGCA CGAATCAAGA TGAACATGCA ATTAAATACT TTGGGCACTG TATTGACCAA    300

ACAGCTTTAT CTCCAAAAGT TAAAGGTCAA TTGAAACAGA AAAAGCTTAT TATGTCTGGA    360

AAAGTATTAA AAGTAATGGT ATCAAATGAC ATTGAACGTA ATCATTTTGA TAAGGCATGT    420

AATGGAAGTC TTATCAAAGC GTTTAGAAAT TGTGGTTTTG ATATCGATAA AATCATATTC    480

GAAACAAATG ATAATGATCA AGAACAAAAC TTAGCTTCTT TAGAAGCACA TATTCAAGAA    540

GAAGACGAAC AAAGTGCACG ATTGGCAACA GAGAAACTTG AAAAAATGAA AGCTGAAAAA    600

GCGAAACAAC AAGATAACAA GCAAAGTGCT GTCGATAAGT GTCAAATTGG TAAGCCGATT    660

CAAATTGAAA ATATTAAACC AATTGAATCT ATTATTGAGG AAGAGTTTAA AGTTGCAATA    720

GAGGGTGTCA TTTTTGATAT AAACTTAAAA GAACTTAAAA GTGGTCGCCA TATCGTAGAA    780

ATTAAAGTGA CTGACTATAC GGACTCTTTA GTTTTAAAAA TGTTTACTCG TAAAAACAAA    840

GATGATTTAG AACATTTTAA AGCGCTAAGT GTTGGTAAAT GGGTTAGGGC TCAAGGTCGT    900

ATTGAAGAAG ATACATTTAT TAGAGATTTA GTTATGATGA TGTCTGATAT TGAAGAGATT    960

AAAAAAGCGA CAAAAAAAGA TAAGGCTGAA GAAAAGCGAG TAGAATTCCA CTTGCATACT   1020

GCAATGAGCC AAATGGATGG TATACCCAAT ATTGGTGCGT ATGTTAAACA GGCAGCAGAC   1080

TGGGGACATC CAGCCATTGC GGTTACAGAC CATAATGTGG TGCAAGCATT TCCAGATGCT   1140

CACGCAGCAG CGGAAAAACA TGGCATTAAA ATGATATACG GTATGGAAGG TATGTTAGTT   1200

GATGATGGTG TTCCGATTGC ATACAAACCA CAAGATGTCG TATTAAAAGA TGCTACTTAT   1260

GTTGTGTTCG ACGTTGAGAC AACTGGTTTA TCAAATCAGT ATGATAAAAT CATCGAGCTT   1320

GCAGCTGTGA AAGTTCATAA CGGTGAAATC ATCGATAAGT TTGAAAGGTT TAGTAATCCG   1380

CATGAACGAT TATCGGAAAC GATTATCAAT TTGACGCATA TTACTGATGA TATGTTAGTA   1440

GATGCCCCTG AGATTGAAGA AGTACTTACA GAGTTTAAAG AATGGGTTGG CGATGCGATA   1500

TTCGTAGCGC ATAATGCTTC GTTTGATATG GGCTTCATCG ATACGGGATA TGAACGTCTT   1560
```

```
GGGTTTGGAC CATCAACGAA TGGTGTTATC GATACTTTAG AATTATCTCG TACGATTAAT    1620

ACTGAATATG GTAAACATGG TTTGAATTTC TTGGCTAAAA AATATGGCGT AGAATTAACG    1680

CAACATCACC GTGCCATTTA TGATACAGAA GCAACAGCTT ACATTTTCAT AAAAATGGTT    1740

CAACAAATGA AAGAATTAGG CGTATTAAAT CATAACGAAA TCAACAAAAA ACTCAGTAAT    1800

GAAGATGCAT ATAAACGTGC AAGACCTAGT CATGTCACAT TAATTGTACA AAACCAACAA    1860

GGTCTTAAAA ATCTATTTAA AATTGTAAGT GCATCATTGG TGAAGTATTT CTACCGTACA    1920

CCTCGAATTC CACGTTCATT GTTAGATGAA TATCGTGAGG GATTATTGGT AGGTACAGCG    1980

TGTGATGAAG GTGAATTATT TACGGCAGTT ATGCAGAAGG ACCAGAGTCA AGTTGAAAAA    2040

ATTGCCAAAT ATTATGATTT TATTGAAATT CAACCACCGG CACTTTATCA AGATTTAATT    2100

GATAGAGAGC TTATTAGAGA TACTGAAACA TTACATGAAA TTTATCAACG TTTAATACAT    2160

GCAGGTGACA CAGCGGGTAT ACCTGTTATT GCGACAGGAA ATGCACACTA TTTGTTTGAA    2220

CATGATGGTA TCGCACGTAA AATTTTAATA GCATCACAAC CCGGCAATCC ACTTAATCGC    2280

TCAACTTTAC CGGAAGCACA TTTTAGAACT ACAGATGAAA TGTTAAACGA GTTTCATTTT    2340

TTAGGTGAAG AAAAAGCGCA TGAAATTGTT GTGAAAAATA CAAACGAATT AGCAGATCGA    2400

ATTGAACGTG TTGTTCCTAT TAAAGATGAA TTATACACAC CGCGTATGGA AGGTGCTAAC    2460

GAAGAAATTA GAGAACTAAG TTATGCAAAT GCGCGTAAAC TGTATGGTGA AGACCTGCCT    2520

CAAATCGTAA TTGATCGATT AGAAAAAGAA TTAAAAAGTA TTATCGGTAA TGGATTTGCG    2580

GTAATTTACT TAATTTCGCA ACGTTTAGTT AAAAAATCAT TAGATGATGG ATACTTAGTT    2640

GGTTCCCGTG GTTCAGTAGG TTCTAGTTTT GTAGCGACAA TGACTGAGAT TACTGAAGTA    2700

AACCCGTTAC CGCCACACTA TATTTGTCCG AACTGTAAAA CGAGTGAATT TTTCAATGAT    2760

GGTTCAGTAG GATCAGGATT TGATTTACCT GATAAGACGT GTGAAACTTG TGGAGCGCCA    2820

CTTATTAAAG AAGGACAAGA TATTCCGTTT GAAAAATTTT TAGGATTTAA GGGAGATAAA    2880

GTTCCTGATA TCGACTTAAA CTTTAGTGGT GAATATCAAC CGAATGCCCA TAACTACACA    2940

AAAGTATTAT TTGGTGAGGA TAAAGTATTC CGTGCAGGTA CAATTGGTAC TGTTGCTGAA    3000

AAGACTGCTT TTGGTTATGT TAAAGGTTAT TTGAATGATC AAGGTATCCA CAAAAGAGGT    3060

GCTGAAATAG ATCGACTCGT TAAAGGATGT ACAGGTGTAC CTGATTACAT GGATATTTAT    3120

GATTTTACGC CGATACAATA TCCTGCCGAT GATCAAAATT CAGCATGGAT GACGACACAT    3180

TTTGATTTCC ATTCTATTCA TGATAATGTA TTAAAACTTG ATATACTTGG ACACGATGAT    3240

CCAACAATGA TTCGTATGCT TCAAGATTTA TCAGGAATTG ATCCAAAAAC AATACCTGTA    3300

GATGATAAAG AAGTTATGCA GATATTTAGT ACACCTGAAA GTTTGGGTGT TACTGAAGAT    3360

GAAATTTTAT GTAAAACAGG TACATTTGGG GTACCGAATT CGGACAGGAT TCGTCGTCAA    3420
```

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```
ATGTTAGAAG ATACAAAGCC AACAACATTT TCTGAATTAG TTCAAATCTC AGGATTATCT        3480

CATGGTACAG ATGTGTGGTT AGGCAATGCT CAAGAATTAA TTAAAACCGG TATATGTGAT        3540

TTATCAAGTG TAATTGGTTG TCGTGATGAT ATCATGGTTT ATTTAATGTA TGCTGGTTTA        3600

GAACCATCAA TGGCTTTTAA AATAATGGAG TCAGTACGTA AAGGTAAAGG TTTAACTGAA        3660

GAAATGATTG AAACGATGAA AGAAAATGAA GTGCCAGATT GGTATTTAGA TTCATGTCTT        3720

AAAATTAAGT ACATATTCCC TAAAGCCCAT GCAGCAGCAT ACGTTTTAAT GGCAGTACGT        3780

ATCGCATATT TCAAAGTACA TCATCCACTT TATTACTATG CATCTTACTT TACAATTCGT        3840

GCGTCAGACT TTGATTTAAT CACGATGATT AAAGATAAAA CAAGCATTCG AAATACTGTA        3900

AAAGACATGT ATTCTCGCTA TATGGATCTA GGTAAAAAAG AAAAAGACGT ATTAACAGTC        3960

TTGGAAATTA TGAATGAAAT GGCGCATCGA GGTTATCGAA TGCAACCGAT TAGTTTAGAA        4020

AAGAGTCAGG CGTTCGAATT TATCATTGAA GGCGATACAC TTATTCCGCC GTTCATATCA        4080

GTGCCTGGGC TTGGCGAAAA CGTTGCGAAA CGAATTGTTG AAGCTCGTGA CGATGGCCCA        4140

TTTTTATCAA AAGAAGATTT AAACAAAAAA GCTGGATTAT ATCAGAAAAT TATTGAGTAT        4200

TTAGATGAGT TAGGCTCATT ACCGAATTTA CCAGATAAAG CTCAACTTTC GATATTTGAT        4260

ATGTAA                                                                   4266
```

The amino acid sequence of the *S. aureus* PolC gene product, Pol III-L is as follows (SEQ. ID. No. 8):

```
Met Thr Glu Gln Gln Lys Phe Lys Val Leu Ala Asp Gln Ile Lys Ile
1               5               10              15

Ser Asn Gln Leu Asp Ala Glu Ile Leu Asn Ser Gly Glu Leu Thr Arg
            20              25              30

Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
        35              40              45

Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
    50              55              60

Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
65              70              75              80

Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His
            85              90              95

Cys Ile Asp Gln Thr Ala Leu Ser Pro Lys Val Lys Gly Gln Leu Lys
            100             105             110

Gln Lys Lys Leu Ile Met Ser Gly Lys Val Leu Lys Val Met Val Ser
        115             120             125
```

```
        Asn Asp Ile Glu Arg Asn His Phe Asp Lys Ala Cys Asn Gly Ser Leu
            130                 135             140

5       Ile Lys Ala Phe Arg Asn Cys Gly Phe Asp Ile Asp Lys Ile Ile Phe
        145                 150             155                 160

        Glu Thr Asn Asp Asn Asp Gln Glu Gln Asn Leu Ala Ser Leu Glu Ala
10                      165                 170             175

        His Ile Gln Glu Glu Asp Glu Gln Ser Ala Arg Leu Ala Thr Glu Lys
                    180                 185             190

15      Leu Glu Lys Met Lys Ala Glu Lys Ala Lys Gln Gln Asp Asn Lys Gln
                195             200             205

        Ser Ala Val Asp Lys Cys Gln Ile Gly Lys Pro Ile Gln Ile Glu Asn
            210             215             220

20      Ile Lys Pro Ile Glu Ser Ile Ile Glu Glu Glu Phe Lys Val Ala Ile
        225                 230             235                 240

        Glu Gly Val Ile Phe Asp Ile Asn Leu Lys Glu Leu Lys Ser Gly Arg
                        245             250             255

25      His Ile Val Glu Ile Lys Val Thr Asp Tyr Thr Asp Ser Leu Val Leu
                    260             265             270

        Lys Met Phe Thr Arg Lys Asn Lys Asp Asp Leu Glu His Phe Lys Ala
30          275             280             285

        Leu Ser Val Gly Lys Trp Val Arg Ala Gln Gly Arg Ile Glu Glu Asp
            290             295             300

35      Thr Phe Ile Arg Asp Leu Val Met Met Met Ser Asp Ile Glu Glu Ile
        305                 310             315                 320

        Lys Lys Ala Thr Lys Lys Asp Lys Ala Glu Glu Lys Arg Val Glu Phe
                    325             330             335

40      His Leu His Thr Ala Met Ser Gln Met Asp Gly Ile Pro Asn Ile Gly
                    340             345             350

        Ala Tyr Val Lys Gln Ala Ala Asp Trp Gly His Pro Ala Ile Ala Val
45              355             360             365

        Thr Asp His Asn Val Val Gln Ala Phe Pro Asp Ala His Ala Ala Ala
            370             375             380

50      Glu Lys His Gly Ile Lys Met Ile Tyr Gly Met Glu Gly Met Leu Val
        385             390             395             400

        Asp Asp Gly Val Pro Ile Ala Tyr Lys Pro Gln Asp Val Val Leu Lys
                        405             410             415

55      Asp Ala Thr Tyr Val Val Phe Asp Val Glu Thr Thr Gly Leu Ser Asn
                    420             425             430

        Gln Tyr Asp Lys Ile Ile Glu Leu Ala Ala Val Lys Val His Asn Gly
60              435             440             445
```

```
      Glu Ile Ile Asp Lys Phe Glu Arg Phe Ser Asn Pro His Glu Arg Leu
          450                 455             460

5     Ser Glu Thr Ile Ile Asn Leu Thr His Ile Thr Asp Asp Met Leu Val
      465             470             475                 480

      Asp Ala Pro Glu Ile Glu Glu Val Leu Thr Glu Phe Lys Glu Trp Val
                  485             490                 495

10    Gly Asp Ala Ile Phe Val Ala His Asn Ala Ser Phe Asp Met Gly Phe
                  500             505                 510

      Ile Asp Thr Gly Tyr Glu Arg Leu Gly Phe Gly Pro Ser Thr Asn Gly
              515                 520             525

15    Val Ile Asp Thr Leu Glu Leu Ser Arg Thr Ile Asn Thr Glu Tyr Gly
          530             535             540

      Lys His Gly Leu Asn Phe Leu Ala Lys Lys Tyr Gly Val Glu Leu Thr
      545             550             555                 560

      Gln His His Arg Ala Ile Tyr Asp Thr Glu Ala Thr Ala Tyr Ile Phe
                  565             570                 575

25    Ile Lys Met Val Gln Gln Met Lys Glu Leu Gly Val Leu Asn His Asn
              580             585             590

      Glu Ile Asn Lys Lys Leu Ser Asn Glu Asp Ala Tyr Lys Arg Ala Arg
          595             600             605

30    Pro Ser His Val Thr Leu Ile Val Gln Asn Gln Gln Gly Leu Lys Asn
          610             615             620

      Leu Phe Lys Ile Val Ser Ala Ser Leu Val Lys Tyr Phe Tyr Arg Thr
35    625             630             635                 640

      Pro Arg Ile Pro Arg Ser Leu Leu Asp Glu Tyr Arg Glu Gly Leu Leu
                  645             650                 655

40    Val Gly Thr Ala Cys Asp Glu Gly Glu Leu Phe Thr Ala Val Met Gln
                  660             665                 670

      Lys Asp Gln Ser Gln Val Glu Lys Ile Ala Lys Tyr Tyr Asp Phe Ile
          675             680                 685

45    Glu Ile Gln Pro Pro Ala Leu Tyr Gln Asp Leu Ile Asp Arg Glu Leu
          690             695                 700

      Ile Arg Asp Thr Glu Thr Leu His Glu Ile Tyr Gln Arg Leu Ile His
50    705             710             715                 720

      Ala Gly Asp Thr Ala Gly Ile Pro Val Ile Ala Thr Gly Asn Ala His
                  725             730                 735

55    Tyr Leu Phe Glu His Asp Gly Ile Ala Arg Lys Ile Leu Ile Ala Ser
              740             745             750

      Gln Pro Gly Asn Pro Leu Asn Arg Ser Thr Leu Pro Glu Ala His Phe
60            755             760             765
```

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```
          Arg Thr Thr Asp Glu Met Leu Asn Glu Phe His Phe Leu Gly Glu Glu
              770             775             780

 5        Lys Ala His Glu Ile Val Val Lys Asn Thr Asn Glu Leu Ala Asp Arg
          785             790             795                 800

          Ile Glu Arg Val Val Pro Ile Lys Asp Glu Leu Tyr Thr Pro Arg Met
                      805             810             815

10        Glu Gly Ala Asn Glu Glu Ile Arg Glu Leu Ser Tyr Ala Asn Ala Arg
                      820             825             830

          Lys Leu Tyr Gly Glu Asp Leu Pro Gln Ile Val Ile Asp Arg Leu Glu
                  835             840             845

.15       Lys Glu Leu Lys Ser Ile Ile Gly Asn Gly Phe Ala Val Ile Tyr Leu
              850             855             860

20        Ile Ser Gln Arg Leu Val Lys Lys Ser Leu Asp Asp Gly Tyr Leu Val
          865             870             875             880

          Gly Ser Arg Gly Ser Val Gly Ser Ser Phe Val Ala Thr Met Thr Glu
                      885             890             895

25        Ile Thr Glu Val Asn Pro Leu Pro Pro His Tyr Ile Cys Pro Asn Cys
                  900             905             910

          Lys Thr Ser Glu Phe Phe Asn Asp Gly Ser Val Gly Ser Gly Phe Asp
              915             920             925

30        Leu Pro Asp Lys Thr Cys Glu Thr Cys Gly Ala Pro Leu Ile Lys Glu
              930             935             940

35        Gly Gln Asp Ile Pro Phe Glu Lys Phe Leu Gly Phe Lys Gly Asp Lys
          945             950             955             960

          Val Pro Asp Ile Asp Leu Asn Phe Ser Gly Glu Tyr Gln Pro Asn Ala
                      965             970             975

40        His Asn Tyr Thr Lys Val Leu Phe Gly Glu Asp Lys Val Phe Arg Ala
                  980             985             990

          Gly Thr Ile Gly Thr Val Ala Glu Lys Thr Ala Phe Gly Tyr Val Lys
                  995             1000            1005

45        Gly Tyr Leu Asn Asp Gln Gly Ile His Lys Arg Gly Ala Glu Ile Asp
              1010            1015            1020

50        Arg Leu Val Lys Gly Cys Thr Gly Val Arg Ala Thr Thr Gly Gln His
          1025            1030            1035            1040

          Pro Gly Gly Ile Ile Val Val Pro Asp Tyr Met Asp Ile Tyr Asp Phe
                      1045            1050            1055

55        Thr Pro Ile Gln Tyr Pro Ala Asp Asp Gln Asn Ser Ala Trp Met Thr
                  1060            1065            1070

          Thr His Phe Asp Phe His Ser Ile His Asp Asn Val Leu Lys Leu Asp
                  1075            1080            1085
60
```

```
        Ile Leu Gly His Asp Asp Pro Thr Met Ile Arg Met Leu Gln Asp Leu
            1090               1095               1100

5       Ser Gly Ile Asp Pro Lys Thr Ile Pro Val Asp Asp Lys Glu Val Met
        1105              1110               1115               1120

        Gln Ile Phe Ser Thr Pro Glu Ser Leu Gly Val Thr Glu Asp Glu Ile
                     1125               1130               1135

10      Leu Cys Lys Thr Gly Thr Phe Gly Val Pro Asn Ser Asp Arg Ile Arg
                 1140               1145               1150

        Arg Gln Met Leu Glu Asp Thr Lys Pro Thr Thr Phe Ser Glu Leu Val
                1155               1160               1165

15      Gln Ile Ser Gly Leu Ser His Gly Thr Asp Val Trp Leu Gly Asn Ala
            1170               1175               1180

        Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly
20      1185               1190               1195               1200

        Cys Arg Asp Asp Ile Met Val Tyr Leu Met Tyr Ala Gly Leu Glu Pro
                     1205               1210               1215

25      Ser Met Ala Phe Lys Ile Met Glu Ser Val Arg Lys Gly Lys Gly Leu
                 1220               1225               1230

        Thr Glu Glu Met Ile Glu Thr Met Lys Glu Asn Glu Val Pro Asp Trp
                1235               1240               1245

30      Tyr Leu Asp Ser Cys Leu Lys Ile Lys Tyr Ile Phe Pro Lys Ala His
            1250               1255               1260

        Ala Ala Ala Tyr Val Leu Met Ala Val Arg Ile Ala Tyr Phe Lys Val
35      1265               1270               1275               1280

        His His Pro Leu Tyr Tyr Tyr Ala Ser Tyr Phe Thr Ile Arg Ala Ser
                     1285               1290               1295

40      Asp Phe Asp Leu Ile Thr Met Ile Lys Asp Lys Thr Ser Ile Arg Asn
                 1300               1305               1310

        Thr Val Lys Asp Met Tyr Ser Arg Tyr Met Asp Leu Gly Lys Lys Glu
                1315               1320               1325

45      Lys Asp Val Leu Thr Val Leu Glu Ile Met Asn Glu Met Ala His Arg
            1330               1335               1340

        Gly Tyr Arg Met Gln Pro Ile Ser Leu Glu Lys Ser Gln Ala Phe Glu
50      1345               1350               1355               1360

        Phe Ile Ile Glu Gly Asp Thr Leu Ile Pro Pro Phe Ile Ser Val Pro
                     1365               1370               1375

55      Gly Leu Gly Glu Asn Val Ala Lys Arg Ile Val Glu Ala Arg Asp Asp
                 1380               1385               1390

        Gly Pro Phe Leu Ser Lys Glu Asp Leu Asn Lys Lys Ala Gly Leu Tyr
                1395               1400               1405

60
```

```
Gln Lys Ile Ile Glu Tyr Leu Asp Glu Leu Gly Ser Leu Pro Asn Leu
    1410                1415                1420

Pro Asp Lys Ala Gln Leu Ser Ile Phe Asp Met
1425                1430                1435
```

This invention also relates to the sequence of the *S. aureus* dnaN gene encoding the beta subunit. The nucleotide sequence is as follows (SEQ. ID. No.9):

```
ATGATGGAAT TCACTATTAA AAGAGATTAT TTTATTACAC AATTAAATGA CACATTAAAA     60

GCTATTTCAC CAAGAACAAC ATTACCTATA TTAACTGGTA TCAAAATCGA TGCGAAAGAA    120

CATGAAGTTA TATTAACTGG TTCAGACTCT GAAATTTCAA TAGAAATCAC TATTCCTAAA    180

ACTGTAGATG GCGAAGATAT TGTCAATATT TCAGAAACAG GCTCAGTAGT ACTTCCTGGA    240

CGATTCTTTG TTGATATTAT AAAAAAATTA CCTGGTAAAG ATGTTAAATT ATCTACAAAT    300

GAACAATTCC AGACATTAAT TACATCAGGT CATTCTGAAT TTAATTTGAG TGGCTTAGAT    360

CCAGATCAAT ATCCTTTATT ACCTCAAGTT TCTAGAGATG ACGCAATTCA ATTGTCGGTA    420

AAAGTACTTA AAAACGTGAT TGCACAAACG AATTTTGCAG TGTCCACCTC AGAAACACGC    480

CCAGTACTAA CTGGTGTGAA CTGGCTTATA CAAGAAAATG AATTAATATG CACAGCGACT    540

GATTCACACC GCTTGGCTGT AAGAAAGTTG CAGTTAGAAG ATGTTTCTGA AAACAAAAAT    600

GTCATCATTC CAGGTAAGGC TTTAGCTGAA TTAAATAAAA TTATGTCTGA CAATGAAGAA    660

GACATTGATA TCTTCTTTGC TTCAAACCAA GTTTTATTTA AAGTTGGAAA TGTGAACTTT    720

ATTTCTCGAT TATTAGAAGG ACATTATCCT GATACAACAC GTTTATTCCC TGAAAACTAT    780

GAAATTAAAT TAAGTATAGA CAATGGGGAG TTTTATCATG CGATTGATCG TGCCTCTTTA    840

TTAGCACGTG AAGGTGGTAA TAACGTTATT AAATTAAGTA CAGGTGATGA CGTTGTTGAA    900

TTATCTTCTA CATCACCAGA AATTGGTACT GTAAAGAAG AAGTTGATGC AAACGATGTT    960

GAAGGTGGTA GCCTGAAAAT TTCATTCAAC TCTAAATATA TGATGGATGC TTTAAAAGCA   1020

ATCGATAATG ATGAGGTTGA AGTTGAATTC TTCGGTACAA TGAAACCATT TATTCTAAAA   1080

CCAAAAGGTG ACGACTCGGT AACGCAATTA ATTTTACCAA TCAGAACTTA CTAA          1134
```

This amino acid sequence of *S. aureus* beta subunit is as follows (SEQ. ID. No. 10):

```
Met Met Glu Phe Thr Ile Lys Arg Asp Tyr Phe Ile Thr Gln Leu Asn
1               5               10                  15

Asp Thr Leu Lys Ala Ile Ser Pro Arg Thr Thr Leu Pro Ile Leu Thr
            20              25                  30
```

```
              Gly Ile Lys Ile Asp Ala Lys Glu His Glu Val Ile Leu Thr Gly Ser
                      35              40                  45

 5            Asp Ser Glu Ile Ser Ile Glu Ile Thr Ile Pro Lys Thr Val Asp Gly
                  50              55                  60

              Glu Asp Ile Val Asn Ile Ser Glu Thr Gly Ser Val Val Leu Pro Gly
              65                  70                  75                  80
10
              Arg Phe Phe Val Asp Ile Ile Lys Lys Leu Pro Gly Lys Asp Val Lys
                              85                  90                  95

15            Leu Ser Thr Asn Glu Gln Phe Gln Thr Leu Ile Thr Ser Gly His Ser
                          100             105                 110

              Glu Phe Asn Leu Ser Gly Leu Asp Pro Asp Gln Tyr Pro Leu Leu Pro
                      115             120                 125

20            Gln Val Ser Arg Asp Asp Ala Ile Gln Leu Ser Val Lys Val Leu Lys
                  130             135                 140

              Asn Val Ile Ala Gln Thr Asn Phe Ala Val Ser Thr Ser Glu Thr Arg
              145             150                 155                 160
25
              Pro Val Leu Thr Gly Val Asn Trp Leu Ile Gln Glu Asn Glu Leu Ile
                          165             170                 175

30            Cys Thr Ala Thr Asp Ser His Arg Leu Ala Val Arg Lys Leu Gln Leu
                          180             185                 190

              Glu Asp Val Ser Glu Asn Lys Asn Val Ile Ile Pro Gly Lys Ala Leu
                      195             200                 205

35            Ala Glu Leu Asn Lys Ile Met Ser Asp Asn Glu Glu Asp Ile Asp Ile
                      210             215                 220

              Phe Phe Ala Ser Asn Gln Val Leu Phe Lys Val Gly Asn Val Asn Phe
              225             230                 235                 240
40
              Ile Ser Arg Leu Leu Glu Gly His Tyr Pro Asp Thr Thr Arg Leu Phe
                          245             250                 255

45            Pro Glu Asn Tyr Glu Ile Lys Leu Ser Ile Asp Asn Gly Glu Phe Tyr
                          260             265                 270

              His Ala Ile Asp Arg Ala Ser Leu Leu Ala Arg Glu Gly Gly Asn Asn
                      275             280                 285

50            Val Ile Lys Leu Ser Thr Gly Asp Asp Val Val Glu Leu Ser Ser Thr
                  290             295                 300

              Ser Pro Glu Ile Gly Thr Val Lys Glu Glu Val Asp Ala Asn Asp Val
              305             310                 315                 320
55
              Glu Gly Gly Ser Leu Lys Ile Ser Phe Asn Ser Lys Tyr Met Met Asp
                          325             330                 335

60            Ala Leu Lys Ala Ile Asp Asn Asp Glu Val Glu Val Glu Phe Phe Gly
                          340             345                 350
```

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```
         Thr Met Lys Pro Phe Ile Leu Lys Pro Lys Gly Asp Asp Ser Val Thr
                 355                 360             365

         Gln Leu Ile Leu Pro Ile Arg Thr Tyr
                 370                 375
```

This invention also relates to the sequence of the *S. aureus* dnaG gene encoding a primase. The nucleotide sequence is as follows (SEQ. ID. No. 11):

```
ATGATAGGTT TGTGTCCTTT TCATGATGAA AAGACACCTT CATTTACAGT TTCTGAAGAT    60

AAACAAATCT GTCATTGTTT TGGTTGTAAA AAAGGTGGCA ATGTTTTTCA ATTTACTCAA   120

GAAATTAAAG ACATATCATT TGTTGAAGCG GTTAAAGAAT TAGGTGATAG AGTTAATGTT   180

GCTGTAGATA TTGAGGCAAC ACAATCTAAC TCAAATGTTC AAATTGCTTC TGATGATTTA   240

CAAATGATTG AAATGCATGA GTTAATACAA GAATTTTATT ATTACGCTTT AACAAAGACA   300

GTCGAAGGCG AACAAGCATT AACATACTTA CAAGAACGTG GTTTTACAGA TGCGCTTATT   360

AAAGAGCGAG GCATTGGCTT TGCACCCGAT AGCTCACATT TTTGTCATGA TTTTCTTCAA   420

AAAAAGGGTT ACGATATTGA ATTAGCATAT GAAGCCGGAT TATTATCACG TAACGAAGAA   480

AATTTCAGTT ATTACGATAG ATTTCGAAAT CGTATTATGT TTCCTTTGAA AAATGCGCAA   540

GGAAGAATTG TTGGATATTC AGGTCGAACA TATACCGGTC AAGAACCAAA ATACCTAAAT   600

AGTCCTGAAA CGCCTATCTT TCAAAAAAGA AAGTTGTTAT ATAACTTAGA TAAAGCACGT   660

AAATCAATTA GAAAATTAGA TGAAATTGTA TTACTAGAAG GTTTTATGGA TGTTATAAAA   720

TCTGATACTG CTGGCTTGAA AAACGTTGTT GCAACAATGG GTACACAGTT GTCAGATGAA   780

CATATTACCT TTATACGAAA GTTAACATCA AATATAACAT TAATGTTTGA TGGGGATTTT   840

GCGGGTAGTG AAGCAACACT TAAAACAGGT CAACATTTGT TACAGCAAGG GCTAAATGTA   900

TTTGTTATAC AATTGCCATC TGGCATGGAT CCGGATGAAT ACATTGGTAA GTATGGCAAC   960

GACGCATTTA CTACTTTTGT AAAAAATGAC AAAAAGTCAT TTGCACATTA TAAAGTAAGT  1020

ATATTAAAAG ATGAAATTGC ACATAATGAC CTTTCATATG AACGTTATTT GAAAGAACTG  1080

AGTCATGACA TTTCACTTAT GAAGTCATCA ATTCTGCAAC AAAAGGCTAT AAATGATGTT  1140

GCGCCATTTT TCAATGTTAG TCCTGAGCAG TTAGCTAACG AAATACAATT CAATCAAGCA  1200

CCAGCCAATT ATTATCCAGA AGATGAGTAT GGCGGTTATG ATGAGTATGG CGGTTATATT  1260

GAACCTGAGC CAATTGGTAT GGCACAATTT GACAATTTGA GCCGTCGAGA AAAAGCGGAG  1320

CGAGCATTTT TAAAACATTT AATGAGAGAT AAAGATACAT TTTTAAATTA TTATGAAAGT  1380

GTTGATAAGG ATAACTTCAC AAATCAGCAT TTTAAATATG TATTCGAAGT CTTACATGAT  1440

TTTTATGCGG AAAATGATCA ATATAATATC AGTGATGCTG TGCAGTATGT TAATTCAAAT  1500
```

```
    GAGTTGAGAG AAACACTAAT TAGCTTAGAA CAATATAATT TGAATGGCGA ACCATATGAA      1560

    AATGAAATTG ATGATTATGT CAATGTTATT AATGAAAAAG GACAAGAAAC AATTGAGTCA      1620

5   TTGAATCATA AATTAAGGGA AGCTACAAGG ATTGGCGATG TAGAATTACA AAAATACTAT      1680

    TTACAGCAAA TTGTTGCTAA GAATAAAGAA CGCATGTAG                            1719
```

The amino acid sequence of primase encoded by *S. aureus* dnaG is as
10   follows (SEQ. ID. No. 12):

```
        Met Ile Gly Leu Cys Pro Phe His Asp Glu Lys Thr Pro Ser Phe Thr
        1           5               10              15

15      Val Ser Glu Asp Lys Gln Ile Cys His Cys Phe Gly Cys Lys Lys Gly
                    20              25              30

        Gly Asn Val Phe Gln Phe Thr Gln Glu Ile Lys Asp Ile Ser Phe Val
                    35              40              45

20      Glu Ala Val Lys Glu Leu Gly Asp Arg Val Asn Val Ala Val Asp Ile
                50              55              60

        Glu Ala Thr Gln Ser Asn Ser Asn Val Gln Ile Ala Ser Asp Asp Leu
25      65              70              75              80

        Gln Met Ile Glu Met His Glu Leu Ile Gln Glu Phe Tyr Tyr Tyr Ala
                        85              90              95

30      Leu Thr Lys Thr Val Glu Gly Glu Gln Ala Leu Thr Tyr Leu Gln Glu
                    100             105             110

        Arg Gly Phe Thr Asp Ala Leu Ile Lys Glu Arg Gly Ile Gly Phe Ala
                    115             120             125

35      Pro Asp Ser Ser His Phe Cys His Asp Phe Leu Gln Lys Lys Gly Tyr
                130             135             140

        Asp Ile Glu Leu Ala Tyr Glu Ala Gly Leu Leu Ser Arg Asn Glu Glu
40      145             150             155             160

        Asn Phe Ser Tyr Tyr Asp Arg Phe Arg Asn Arg Ile Met Phe Pro Leu
                        165             170             175

45      Lys Asn Ala Gln Gly Arg Ile Val Gly Tyr Ser Gly Arg Thr Tyr Thr
                    180             185             190

        Gly Gln Glu Pro Lys Tyr Leu Asn Ser Pro Glu Thr Pro Ile Phe Gln
                195             200             205

50      Lys Arg Lys Leu Leu Tyr Asn Leu Asp Lys Ala Arg Lys Ser Ile Arg
                210             215             220

        Lys Leu Asp Glu Ile Val Leu Leu Glu Gly Phe Met Asp Val Ile Lys
55      225             230             235             240

        Ser Asp Thr Ala Gly Leu Lys Asn Val Val Ala Thr Met Gly Thr Gln
                        245             250             255
```

```
        Leu Ser Asp Glu His Ile Thr Phe Ile Arg Lys Leu Thr Ser Asn Ile
                    260             265                 270

  5     Thr Leu Met Phe Asp Gly Asp Phe Ala Gly Ser Glu Ala Thr Leu Lys
                    275             280             285

        Thr Gly Gln His Leu Leu Gln Gln Gly Leu Asn Val Phe Val Ile Gln
            290             295             300
 10
        Leu Pro Ser Gly Met Asp Pro Asp Glu Tyr Ile Gly Lys Tyr Gly Asn
        305             310             315                 320

 15     Asp Ala Phe Thr Thr Phe Val Lys Asn Asp Lys Lys Ser Phe Ala His
                    325             330             335

        Tyr Lys Val Ser Ile Leu Lys Asp Glu Ile Ala His Asn Asp Leu Ser
                    340             345             350

 20     Tyr Glu Arg Tyr Leu Lys Glu Leu Ser His Asp Ile Ser Leu Met Lys
                355             360             365

        Ser Ser Ile Leu Gln Gln Lys Ala Ile Asn Asp Val Ala Pro Phe Phe
            370             375             380
 25
        Asn Val Ser Pro Glu Gln Leu Ala Asn Glu Ile Gln Phe Asn Gln Ala
        385             390             395                 400

        Pro Ala Asn Tyr Tyr Pro Glu Asp Glu Tyr Gly Gly Tyr Asp Glu Tyr
 30                     405             410                 415

        Gly Gly Tyr Ile Glu Pro Glu Pro Ile Gly Met Ala Gln Phe Asp Asn
                    420             425             430

 35     Leu Ser Arg Arg Glu Lys Ala Glu Arg Ala Phe Leu Lys His Leu Met
                435             440             445

        Arg Asp Lys Asp Thr Phe Leu Asn Tyr Tyr Glu Ser Val Asp Lys Asp
            450             455             460
 40
        Asn Phe Thr Asn Gln His Phe Lys Tyr Val Phe Glu Val Leu His Asp
        465             470             475                 480

        Phe Tyr Ala Glu Asn Asp Gln Tyr Asn Ile Ser Asp Ala Val Gln Tyr
 45                     485             490                 495

        Val Asn Ser Asn Glu Leu Arg Glu Thr Leu Ile Ser Leu Glu Gln Tyr
                    500             505             510

 50     Asn Leu Asn Gly Glu Pro Tyr Glu Asn Glu Ile Asp Asp Tyr Val Asn
                515             520             525

        Val Ile Asn Glu Lys Gly Gln Glu Thr Ile Glu Ser Leu Asn His Lys
            530             535             540
 55
        Leu Arg Glu Ala Thr Arg Ile Gly Asp Val Glu Leu Gln Lys Tyr Tyr
        545             550             555                 560

        Leu Gln Gln Ile Val Ala Lys Asn Lys Glu Arg Met
 60                     565             570
```

Fragments of the above polypeptides or proteins are also encompassed by the present invention.

Suitable fragments can be produced by several means. In the first,

5   subclones of the gene encoding the protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for activity according to the procedures described below.

10   As an alternative, fragments of replication proteins can be produced by digestion of a full-length replication protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave replication proteins at different sites based on the amino acid sequence of the protein. Some of the fragments that result from proteolysis may

15   be active.

In another approach, based on knowledge of the primary structure of the protein, fragments of a replication protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for

20   increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences of replication proteins being produced. Alternatively, subjecting a full length replication protein to high temperatures and pressures will produce fragments. These fragments can then be

25   separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end

30   of the protein which cotranslationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. Nos. 1, 3, 5, 7, 9, or 11 under stringent conditions such as those characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and

5   remaining bound when subject to washing the SSC buffer at a temperature of 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The proteins or polypeptides of the present invention are preferably

10   produced in purified form (preferably at least 80%, more preferably 90%, pure) by conventional techniques. Typically, the proteins or polypeptides of the present invention is secreted into the growth medium of recombinant host cells. Alternatively, the proteins or polypeptides of the present invention are produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell

15   (e.g., E. coli) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to purification procedures such as ammonium sulfate precipitation, gel filtration, ion exchange chromatography, FPLC, and HPLC.

The DNA molecule encoding replication polypeptides or proteins

20   derived from Gram positive bacteria can be incorporated in cells using conventional recombinant DNA technology. Generally, this involved inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector

25   contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA

30   ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral

5   vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors .such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see

10   F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard

15   cloning procedures in the art, as described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host

20   cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression

25   elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

30   Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from

those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further procaryotic promoters are not recognized and do not function in eucaryotic cells.

5   Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the same codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promotor, *trp* promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp, pro*, etc.,

are under different controls. Additionally, the cell may carry the gene for a heterologous RNA polymerase such as from phage T7. Thus, a promoter specific for T7 RNA polymerase is used. The T7 RNA polymerase may be under inducible control.

5   Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong"

10   transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, an SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage

15   lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

         Once the isolated DNA molecule encoding a replication polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a

20   host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, viruses, yeast, mammalian cells, insects, plants, and the like.

         The invention provides efficient methods of identifying

25   pharmacological agents or lead compounds for agents active at the level of a replication protein function, particularly DNA replication. Generally, these screening methods involve assaying for compounds which interfere with the replication activity. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the

30   pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Target therapeutic indications are

limited only in that the target cellular function be subject to modulation, usually inhibition, by disruption of a replication activity or the formation of a complex comprising a replication protein and one or more natural intracellular binding targets. Target indications may include arresting cell growth or causing cell death resulting in

5   recovery from the bacterial infection in animal studies.

A wide variety of assays for activity and binding agents are provided, including DNA synthesis, ATPase, clamp loading onto DNA, protein-protein binding assays, immunoassays, cell based assays, etc. The replication protein compositions, used to identify pharmacological agents, are in isolated, partially pure or pure form

10   and are typically recombinantly produced. The replication protein may be part of a fusion product with another peptide or polypeptide (e.g., a polypeptide that is capable of providing or enhancing protein-protein binding, stability under assay conditions (e.g., a tag for detection or anchoring), etc.). The assay mixtures comprise a natural intracellular replication protein binding target such as DNA, another protein, NTP, or

15   dNTP. For binding assays, while native binding targets may be used, it is frequently preferred to use portions (e.g., peptides, nucleic acid fragments) thereof so long as the portion provides binding affinity and avidity to the subject replication protein conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Generally, a plurality of assay mixtures are run in parallel

20   with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control (i.e. at zero concentration or below the limits of assay detection). Additional controls are often present such as a positive control, a dose response curve, use of known inhibitors, use of control heterologous proteins, etc. Candidate agents encompass

25   numerous chemical classes, though typically they are organic compounds; preferably they are small organic compounds and are obtained from a wide variety of sources, including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral proteins (e.g., albumin, detergents, etc.), which may be used to facilitate

30   optimal binding and/or reduce nonspecific or background interactions, etc. Also reagents that otherwise improve the efficiency of the assay (e.g., protease inhibitors, nuclease inhibitors, antimicrobial agents, etc.) may be used.

The invention provides replication protein specific assays and the binding agents including natural intracellular binding targets such as other replication proteins, etc., and methods of identifying and making such agents, and their use in a variety of diagnostic and therapeutic applications, especially where disease is associated with excessive cell growth. Novel replication protein-specific binding agents include replication protein-specific antibodies and other natural intracellular binding agents identified with assays such as one- and two-hybrid screens, non-natural intracellular binding agents identified in screens of chemical libraries, etc.

Generally, replication protein-specificity of the binding agent is shown by binding equilibrium constants. Such agents are capable of selectively binding a replication protein (i.e., with an equilibrium constant at least about 10^7 M^{-1}, preferably, at least about 10^8 M^{-1}, more preferably, at least about 10^9 M^{-1}). A wide variety of cell-based and cell-free assays may be used to demonstrate replication protein-specific activity, binding, gel shift assays, immunoassays, etc.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the replication protein specifically binds the cellular binding target, portion, or analog. The mixture of components can be added in any order that provides for the requisite bindings. Incubations may be performed at any temperature which facilitates optimal binding, typically between 4 and 40°C, more commonly between 15° and 40°C. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening, and are typically between 0.1 and 10 hours, preferably less than 5 hours, more preferably less than 2 hours.

After incubation, the presence or absence of activity or specific binding between the replication protein and one or more binding targets is detected by any convenient way. For cell-free activity and binding type assays, a separation step may be used to separate the activity product or the bound from unbound components. Separation may be effected by precipitation (e.g., immunoprecipitation), immobilization (e.g., on a solid substrate such as a microtiter plate), etc., followed by washing. Many assays that do not require separation are also possible such as use of europium conjugation in proximity assays or a detection system that is dependent on a product or loss of substrate.

Detection may be effected in any convenient way. For cell-free activity and binding assays, one of the components usually comprises or is coupled to a label. A wide variety of labels may be employed – essentially any label that provides for detection of DNA product, loss of DNA substrate, conversion of a

5   nucleotide substrate, or bound protein is useful. The label may provide for direct detection such as radioactivity, fluorescence, luminescence, optical, or electron density, etc. or indirect detection such as an epitope tag, an enzyme, etc. The label may be appended to the protein (e.g., a phosphate group comprising a radioactive isotope of phosphorous), or incorporated into the DNA substrate or the protein

10   structure (e.g., a methionine residue comprising a radioactive isotope of sulfur.) A variety of methods may be used to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate, or a portion of the bound complex containing the label may be separated from the solid substrate, and thereafter the label detected. Labels may be

15   directly detected through optical or electron density, radioactive emissions, nonradiative energy transfer, fluorescence emission, etc. or indirectly detected with antibody conjugates, etc. For example, in the case of radioactive labels, emissions may be detected directly (e.g., with particle counters) or indirectly (e.g., with scintillation cocktails and counters).

20   The present invention identifies the type of replication system that Gram positive bacteria utilize. Specifically, the replicase is comprised of a DNA polymerase III-type enzyme and it is made functional by other components that are needed for processive function. These components include a sliding clamp and a clamp loader. Hence, Gram negative bacteria do not utilize the replication strategies

25   exemplified by one and two component processive replicases.

The present invention also identifies, partially purifies, and characterizes a second Pol III-type replicase. The polymerase of the second Pol III-type enzyme, termed Pol III-2, behaves like Pol III-L in that it also functions with the clamp and clamp loader components.

30   This invention also expresses and purifies a protein from a Gram positive bacteria that is homologous to the *E. coli* beta subunit. The invention demonstrates that it behaves like a circular protein. Further, this invention shows that

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the beta subunit from a Gram positive bacteria is functional with both Pol III-L from a

Gram positive bacteria and with DNA polymerase III from a Gram negative bacteria.

This result can be explained by an interaction between the clamp and the polymerase

that has been conserved during the evolutionary divergence of Gram positive and

5   Gram negative cells. A chemical inhibitor that would disrupt this interaction would

be predicted to have a broad spectrum of antibiotic activity, shutting down replication

in gram negative and gram positive cells alike. This assay, and others based on this

interaction, can be devised to screen chemicals for such inhibition. Further, since all

the proteins in this assay are highly overexpressed through recombinant techniques,

10   sufficient quantities of the protein reagents can be obtained for screening hundreds of

thousands of compounds.

The present invention provides methods by which replication proteins

from a Gram positive bacteria are used to discover new pharmaceutical agents. The

function of replication proteins is quantified in the presence of different chemical

15   compounds. A chemical compound that inhibits the function is a candidate antibiotic.

Some replication proteins from a Gram positive bacteria and from a Gram negative

bacteria can be interchanged for one another. Hence, they can function as mixtures.

Reactions that assay for the function of enzyme mixtures consisting of proteins from

Gram positive bacteria and from Gram negative bacteria can also be used to discover

20   drugs. Suitable *E. coli* replication proteins are the subunits of its Pol III holoenzyme

which are described in U.S. Patent Nos. 5,583,026 and 5,668,004, which are hereby

incorporated by reference.

The methods described here to obtain genes, and the assays

demonstrating activity behavior of *S. aureus* are likely to generalize to all members of

25   the *Staphylococcus* genus and to all Gram positive bacteria.

The present invention describes a method to identify chemicals that

inhibit the activity of the Pol III-2 and/or Pol III-L. This method involves contacting

primed DNA with the DNA polymerase in the presence of the candidate

pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The

30   reaction mixture is subjected to conditions effective to achieve nucleic acid

polymerization in the absence of the candidate pharmaceutical and the presence or

absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit to stimulate Pol III-2 and/or Pol III-L. This method involves contacting a linear primed DNA with a beta subunit and a DNA polymerase in the presence of the candidate compound, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions which, in the absence of the candidate compound, would affect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The beta subunit and/or the DNA polymerase are derived from a Gram positive bacterium.

The present invention also describes a method to identify candidate pharmaceuticals that inhibit the activity of a gamma complex (or a subunit or subassembly of the gamma complex) and a beta subunit in stimulating either Pol III-2 or Pol III-L. The method includes contacting a primed DNA (which may be coated with SSB) with a DNA polymerase, a beta subunit, and a gamma complex (or subunit or subassembly of the gamma complex) in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions which in the absence of the candidate pharmaceutical would effect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The DNA polymerase, the beta subunit, and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit and a DNA polymerase to interact physically. This method involves contacting the beta subunit with the DNA polymerase in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA polymerase and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta unit and the DNA

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polymerase. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the DNA polymerase. The DNA polymerase and/or the beta subunit are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that

5   inhibit the ability of a beta subunit and a gamma complex (or a subunit or subassembly of the gamma complex) to interact. This method includes contacting the beta subunit with the gamma complex (or subunit or subassembly of the gamma complex) in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or

10   the subunit or subassembly of the gamma complex) and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta subunit and the gamma complex (or the subunit or subassembly of the gamma complex). The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the gamma

15   complex (or the subunit or subassembly of the gamma complex) . The beta subunit and/or the gamma complex or subunit thereof is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a gamma complex (or a subassembly of the gamma complex) to

20   assemble a beta subunit onto a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) with the gamma complex (or the subassembly thereof) and the beta subunit in the presence of the candidate pharmaceutical, and ATP or dATP to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or subassembly)

25   assembles the beta subunit on the DNA molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit and/or the gamma complex are derived from a Gram positive bacterium.

30   The present invention describes a method to identify chemicals that inhibit the ability of a gamma complex (or a subunit(s) of the gamma complex) to disassemble a beta subunit from a DNA molecule. This method comprises contacting

a DNA molecule onto which the beta subunit has been assembled in the presence of the candidate pharmaceutical, to form a reaction mixture. The reaction mixture is subjected to conditions under which the gamma complex (or a subunit(s) or subassembly of the gamma complex) disassembles the beta subunit from the DNA

5   molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the presence of the beta subunit on the DNA molecule. The beta subunit and/or the gamma complex are derived from a Gram positive bacterium.

10   The present invention describes a method to identify chemicals that dissassemble a beta subunit from a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) upon which the beta subunit has been assembled (e.g., by action of the gamma complex) with the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA

15   molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit is derived from a Gram positive bacterium.

            The present invention describes a method to identify chemicals that inhibit the dATP/ATP binding activity of a gamma complex or a gamma complex

20   subunit (e.g., gamma subunit). This method includes contacting the gamma complex (or the gamma complex subunit) with dATP/ATP either in the presence or absence of a DNA molecule and/or the beta subunit in the presence of the candidate pharmaceutical to form a reaction. The reaction mixture is subjected to conditions in which the gamma complex (or the subunit of gamma complex) interacts with

25   dATP/ATP in the absence of the candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP is bound to the gamma complex (or the subunit of gamma complex) in the presence of the candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of hydrolysis. The gamma complex and/or the beta subunit is derived from a Gram positive bacterium.

30   The present invention describes a method to identify chemicals that inhibit the dATP/ATPase activity of a gamma complex or a gamma complex subunit (e.g., the gamma subunit). This method involves contacting the gamma complex (or

the gamma complex subunit) with dATP/ATP either in the presence or absence of a

DNA molecule and/or a beta subunit in the presence of the candidate pharmaceutical

to form a reaction mixture. The reaction mixture is subjected to conditions in which

the gamma subunit (or complex) hydrolyzes dATP/ATP in the absence of the

5   candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP was

hydrolyzed. Suitable candidate pharmaceuticals are identified by the absence of

hydrolysis. The gamma complex and/or the beta subunit is derived from a Gram

positive bacterium.

            The present invention describes methods to identify chemicals that

10   inhibit the activity of a DNA polymerase encoded by either the dnaE gene or PolC

gene. These methods are as follows.

            1)   Contacting a primed DNA molecule with the encoded product

of the dnaE gene or PolC gene in the presence of the candidate pharmaceutical, and

dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is

15   subjected to conditions, which in the absence of the candidate pharmaceutical, affect

nucleic acid polymerization and the presence or absence of the extension product in

the reaction mixture is analyzed. The candidate pharmaceutical is detected by the

absence of extension product. The protein encoded by the dnaE gene and PolC gene

is derived from a Gram positive bacterium.

20   2)   Contacting a linear primed DNA molecule with a beta subunit

and the encoded product of dnaE or PolC in the presence of the candidate

pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The

reaction mixture is subjected to conditions, which in the absence of the candidate

pharmaceutical, affect nucleic acid polymerization, and the presence or absence of the

25   extension product in the reaction mixture is analyzed. The candidate pharmaceutical

is detected by the absence of extension product. The protein encoded by the dnaE

gene and PolC gene is derived from a Gram positive bacterium.

            3)   Contacting a circular primed DNA molecule (may be coated

with SSB) with a gamma complex, a beta subunit and the encoded product of a dnaE

30   gene or PolC gene in the presence of the candidate pharmaceutical, and dNTPs (or

modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to

conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid

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polymerization, and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The protein encoded by the dnaE gene and PolC gene, the beta subunit, and/or the gamma complex are derived from a Gram positive bacterium.

5   4)   Contacting a beta subunit with the product encoded by a dnaE gene or PolC gene in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is then analyzed for interaction between the beta subunit and the product encoded by the dnaE gene or PolC gene. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and

10   the product encoded by the dnaE gene or PolC gene. The beta subunit and/or the protein encoded by the dnaE gene and PolC gene is derived from a Gram positive bacterium.

The present invention discloses a method to identify chemicals that inhibit a DnaB helicase. The method includes contacting the DnaB helicase with a

15   DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support helicase activity in the absence of the candidate pharmaceutical. The DNA duplex molecule in the reaction mixture is analyzed for whether it is converted to ssDNA. The candidate

20   pharmaceutical is detected by the absence of conversion of the duplex DNA molecule to the ssDNA molecule. The DnaB helicase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the nucleoside or deoxynucleoside triphosphatase activity of a DnaB helicase.

25   The method includes contacting the DnaB helicase with a DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support nucleoside or deoxynucleoside triphosphatase activity of the DnaB helicase in the absence of the

30   candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of conversion of nucleoside or deoxynucleoside triphosphate to nucleoside or

deoxynucleoside diphosphate. The DnaB helicase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit a primase. The method includes contacting primase with a ssDNA molecule in

5   the presence of a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support primase activity (e.g., the presence of nucleoside or deoxynucleoside triphosphates, appropriate buffer, presence or absence of DnaB protein) in the absence of the candidate pharmaceutical. Suitable candidate pharmaceuticals are identified by the absence of primer formation detected either

10   directly or indirectly. The primase is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a primase and the protein encoded by a DnaB gene to interact. This method includes contacting the primase with the protein encoded by the DnaB gene in the presence of the candidate pharmaceutical to form a reaction mixture. The

15   reaction mixture is subjected to conditions under which the primase and the protein encoded by the DnaB gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the primase and the protein encoded by the DnaB gene. The candidate pharmaceutical is detected by the absence of interaction between the primase and the protein encoded by the DnaB

20   gene. The primase and/or the DnaB gene are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a protein encoded by a DnaB gene to interact with a DNA molecule. This method includes contacting the protein encoded by the DnaB gene

25   with the DNA molecule in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA molecule and the protein encoded by the DnaB gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the protein encoded by the DnaB gene and the DNA molecule. The

30   candidate pharmaceutical is detected by the absence of interaction between the DNA molecule and the protein encoded by the DnaB gene. The DnaB gene is derived from a Gram positive bacterium.

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EXAMPLES

Example 1 -   Materials

5   Labeled deoxy- and ribonucleoside triphosphates were from Dupont-
New England Nuclear; unlabelled deoxy- and ribonucleoside triphosphates were from
Pharmacia-LKB; *E. coli* replication proteins were purified as described, alpha,
epsilon, gamma, and tau (Studwell, et al., "Processive Replication is Contingent on
the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol. Chem.,
10   265:1171-1178 (1990), which is hereby incorporated by reference), beta (Kong, et. al,
"Three Dimensional Structure of the Beta Subunit of *Escherichia coli* DNA
Polymerase III Holoenzyme: A Sliding DNA Clamp," Cell, 69:425-437 (1992),
which is hereby incorporated by reference), delta and delta prime (Dong, et. al.,
"DNA Polymerase III Accessory Proteins. I. *HolA* and *holB* Encoding δ and δ'," J.
15   Biol. Chem., 268:11758-11765 (1993), which is hereby incorporated by
reference), chi and psi (Xiao, et. al., "DNA Polymerase III Accessory Proteins. III.
HolC and *holD* Encoding chi and psi," J. Biol. Chem., 268:11773-11778 (1993),
which is hereby incorporated by reference), theta (Studwell-Vaughan, et al., "DNA
Polymerase III Accessory Proteins. V. Theta Encoded by *holE*," J. Biol. Chem.,
20   268:11785-11791 (1993), which is hereby incorporated by reference), and SSB
(Weiner, et. al., "The Deoxyribonucleic Acid Unwinding Protein of *Escherichia coli*,"
J. Biol. Chem., 250:1972-1980 (1975), which is hereby incorporated by reference). *E.
coli* Pol III core, and gamma complex (composed of subunits: gamma, delta, delta
prime, chi, and psi) were reconstituted as described in Onrust, et. al., "Assembly of a
25   Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and
Sliding Clamps in One Holoenzyme Particle. I. Organization of the Clamp Loader,"
J. Biol. Chem., 270:13348-13357 (1995), which is hereby incorporated by reference.
Pol III* was reconstituted and purified as described in Onrust, et. al., "Assembly of a
Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and
30   Sliding Clamps in One Holoenzyme Particle. III. Interface Between Two
Polymerases and the Clamp Loader," J. Biol. Chem., 270:13366-13377 (1995), which
is hereby incorporated by reference. Protein concentrations were quantitated by the
Protein Assay (Bio-Rad) method using bovine serum albumin (BSA) as a standard.

DNA oligonucleotides were synthesized by Oligos etc. Calf thymus DNA was from Sigma. Buffer A is 20 mM Tris-HCl (pH=7.5), 0.5 mM EDTA, 2 mM DTT, and 20% glycerol. Replication buffer is 20 mM Tris-Cl (pH 7.5), 8 mM $MgCl_2$, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, 0.5 mM ATP, 3 mM each dCTP, dGTP,

5   dATP, and 20 µM [α-^{32}P]dTTP. P-cell buffer was 50 mM potassium phosphate (pH 7.6), 5 mM DTT, 0.3 mM EDTA, 20% glycerol. T.E. buffer is 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Cell lysis buffer was 50 mM Tris-HCl (pH 8.0) 10 % sucrose, 1 M NaCl, 0.3 mM spermidine.

10   **Example 2 -   Calf Thymus DNA Replication Assays**

These assays were used in the purification of DNA polymerases from *S. aureus* cell extracts. Assays contained 2.5 µg activated calf thymus DNA in a final volume of 25 µl replication buffer. An aliquot of the fraction to be assayed was added

15   to the assay mixture on ice followed by incubation at 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen, et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference.

20   **Example 3 -   PolydA-oligodT Replication Assays**

PolydA-oligodT was prepared as follows. PolydA of average length 4500 nucleotides was purchased from SuperTecs. OligodT35 was synthesized by Oligos etc. 145 ul of 5.2 mM (as nucleotide) polydA and 22 µl of 1.75 mM (as

25   nucleotide) oligodT were mixed in a final volume of 2100 µl T.E. buffer (ratio as nucleotide was 21:1 polydA to oligodT). The mixture was heated to boiling in a 1 ml eppindorf tube, then removed and allowed to cool to room temperature. Assays were performed in a final volume of 25 µl 20 mM Tris-Cl (pH 7.5), 8 mM $MgCl_2$, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, containing 20 µM [α-^{32}P]dTTP

30   and 0.36 µg polydA-oligodT. Proteins were added to the reaction on ice, then shifted to 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen, et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which

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Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference.

Example 4 -   **Singly Primed M13mp18 ssDNA Replication Assays**

M13mp18 was phenol extracted from phage and purified by two successive bandings (one downward and one upward) in cesium chloride gradients. M13mp18 ssDNA was singly primed with a DNA 30mer (map position 6817-6846) as described in Studwell, et al. "Processive Replication is Contingent on the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol. Chem., 265:1171-1178 (1990), which is hereby incorporated by reference. Replication assays contained 72 ng of singly primed M13mp18 ssDNA in a final volume of 25 µl of replication buffer. Other proteins added to the assay, and their amounts, are indicated in the Brief Description of the Drawings. Reactions were incubated for 5 min. at 37°C and then were quenched upon adding an equal volume of 1% SDS and 40 mM EDTA. DNA synthesis was quantitated using DE81 paper as described in Rowen, et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference, and product analysis was performed in a 0.8% native agarose gel followed by autoradiography.

Example 5 -   **Genomic *Staphylococcus aureus* DNA**

Two strains of *S. aureus* were used. For PCR of the first fragment of the dnaX gene sequence, the strain was ATCC 25923. For all other work the strain was strain 4220 (a gift of Dr. Pat Schlievert, University of Minnisota). This strain lacks a gene needed for producing toxic shock (Kreiswirth, et al., "The Toxic Shock Syndrome Exotoxin Structural Gene is Not Detectably Transmitted by a Prophage," Nature, 305:709-712 (1996) and Balan, et al., "Autocrine Regulation of Toxin Synthesis by *Staphylococcus aureus*," Proc. Natl. Acad. Sci. USA, 92:1619-1623 (1995), which are hereby incorporated by reference). *S. aureus* cells were grown overnight at 37°C in LB containing 0.5% glucose. Cells were collected by centrifugation (24 g wet weight). Cells were resuspended in 80 ml solution I (50 mM

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glucose, 10 mM EDTA, 25 mM Tris-HCL (pH 8.0)). Then, SDS and NaOH were

added to 1% and 0.2 N, respectively, followed by incubation at 65°C for 30 min. to

lyse the cells. 68.5 ml of 3 M sodium acetate (pH 5.0) was added followed by

centrifugation at 12,000 rpm for 30 min. The supernatant was discarded and the pellet

5   was washed twice with 50 ml of 6M urea, 10 mM Tris-HCL (pH 7.5), 1 mM EDTA)

using a dounce homogenizer. After each wash, the resuspended pellet was collected

by centrifugation (12,000 rpm for 20 min.). After the second wash, the pellet was

resuspended in 50 ml 10 mM T.E. buffer using a dounce homogenizer and then

incubated for 30 min. at 65°C. The solution was centrifuged at 12,000 rpm for 20

10   min., and the viscous supernatant was collected. 43.46 g $CsCl_2$ was added to the 50

ml of supernatant (density between 1.395-1.398) and poured into two 35 ml quick seal

ultracentrifuge tubes (tubes were completely filled using the same density of $CsCl_2$ in

T.E.). To each tube was added 0.5 ml of a 10 mg/ml stock of ethidium bromide.

Tubes were spun at 55,000 rpm for 18 h at 18°C in a Sorvall TV860 rotor. The band

15   of genomic DNA was extracted using a syringe and needle. Ethidium bromide was

removed using two butanol extractions and then dialyzed against 4 l of T.E. at pH 8.0

overnight. The DNA was recovered by ethanol precipitation and then resuspended in

T.E. buffer (1.7 mg total) and stored at -20°C.

20   **Example 6 -   Cloning and Purification of *S. aureus* Pol III-L Holoenzyme**

               To further characterize the mechanism of DNA replication in *S.*

aureus, large amounts of its replication proteins were produced through use of the

genes. The PolC gene encoding *S. aureus* Pol III-L holoenzyme has been sequenced

25   and expressed in *E. coli* (Pacitti, et. al., "Characterization and Overexpression of the

Gene Encoding *Staphylococcus aureus* DNA Polymerase III," Gene, 165:51-56

(1995), which is hereby incorporated by reference). The previous work utilized a

pBS[KS] vector for expression in which the *E. coli* RNA polymerase is used for gene

transcription. In the earlier study, the *S. aureus* Pol III gene was precisely cloned at

30   the 5' end encoding the N-terminus, but the amount of the gene that remained past the

3' end was not disclosed and the procedure for subcloning the gene into the expression

vector was only briefly summarized. Furthermore, the previous study does not show

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the level of expression of the *S. aureus* Pol III, nor the amount of *S. aureus* Pol III-L

that is obtained from the induced cells. Since the previously published procedure

could not be repeated and the efficiency of the expression vector could not be

assessed, another strategy outlined below had to be developed.

5   The isolated Pol III gene was cloned into a vector that utilizes T7 RNA

polymerase for transcription as this process generally expresses a large amount of

protein. Hence, the *S. aureus* PolC gene was cloned precisely into the start codon at

the NdeI site downstream of the T7 promotor in a pET vector . As the PolC gene

contains an internal NdeI site, the entire gene could not be amplified and placed it into

10   the NdeI site of a pET vector. Hence, a three step cloning strategy that yielded the

desired clone was devised (See Figure 1). These attempts were quite frustrating

initially as no products of cloning in standard *E. coli* strains such as DH5alpha, a

typical laboratory strain for preparation of DNA, could be obtained. Finally, a cell

that was mutated in several genes affecting DNA stability was useful in obtaining the

15   desired products of cloning.

         In brief, the cloning strategy required use of another expression vector

(called pET1137kDa) in which the 37 kDa subunit of human RFC, the clamp loader

of the human replication system, had been cloned into the pET11 vector. The gene

encoding the 37kDa subunit contains an internal NsiI site, which was needed for the

20   precise cloning of the isolated PolC gene. This three step strategy is shown in

Figure 1. In the first step, an approximately 2.3 kb section of the 5' section of the

gene (encoding the N-terminus of Pol III-L) was amplified using the polymerase

chain reaction (PCR). Primers were: upstream

5'-GGTGGTAATTGTCTTG<u>CATATG</u>ACAGAGC-3' (SEQ. ID. No. 13);

25   downstream 5'-AGCGATTAAGTGGATTGCCGGGTTGTGATG C-3' (SEQ. ID.

No. 14). Amplification was performed using 500 ng genomic DNA, 0.5 mM EDTA,

1 μM of each primer, 1mM MgSO$_4$, 2 units vent DNA polymerase (New England

Biolabs) in 100 μl of vent buffer (New England Biolabs). Forty cycles were

performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.; 72°C, 2.5

30   min. The product was digested with NdeI (underlined in the upstream primer) and

NsiI (an internal site in the product) and the approximately 1.8 kb fragment was gel

purified. A pET11 vector containing as an insert the 37 kDa subunit of human

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replication factor C (pET1137kDa) was digested with NdeI and NsiI and gel purified. The PCR fragment was ligated into the digested pET1137kDa vector and the ligation reaction was transformed into Epicurean coli supercompetent SURE 2 cells (Stratagene) and colonies were screened for the correct chimera (pET11PolC1) by

5   examining minipreps for proper length and correct digestion products using NdeI and NsiI. In the second step, an approximately 2076 bp fragment containing the DNA encoding the C-terminus of Pol III-L holoenzyme was amplified using as primers: upstream 5'-AGCATCACAACCCGGCAATCCACTTAATCG C-3' (SEQ. ID. No. 15); downstream, 5'-GACTACG<u>CCATGGG</u>CATTAAATAAATACC-3' (SEQ. ID.

10   No. 16). The amplification cycling scheme was as described above except the elongation step at 72°C was for 2 min. The product was digested with BamHI (underlined in the downstream primer) and NsiI (internal to the product) and the approximately 480 bp product was gel purified and ligated into the pET11PolC1 that had been digested with NsiI/BamHI and gel purified (ligated product is pET11PolC2).

15   To complete the expression vector, an approximately 2080 bp PCR product was amplified over the two NsiI sites internal to the gene using the following primers: upstream 5'-GAAG<u>AT GCA T</u>ATAAACGTGCA AGACCTAGT C-3' (SEQ. ID. No. 17), downstream 5'-GTCTGACGCACGAATTGTAAAGTAAG<u>ATGCAT</u>A G-3' (SEQ. ID. No. 18). The amplification cycling scheme was as described above except

20   the 72°C elongation step was 2 min. The PCR product, and the pET11PolC2 vector, were digested with NsiI and gel purified. The ligation mixture was transformed as described above and colonies were screened for the correct chimera (pET11PolC).

To express Pol III-L holoenzyme, the pET11PolC plasmid was transformed into *E. coli* strain BL21(DE3). 24 L of *E. coli* BL21(DE3)pET11PolC

25   were grown in LB media containing 50 μg/ml ampicillin at 37°C to an OD of 0.7 and then the temperature was lowered to 15°C. Cells were then induced for Pol III-L expression upon addition of 1 mM IPTG to produce the T7 RNA polymerase needed to transcribe PolC holoenzyme. This step was followed by further incubation at 15°C for 18 h. Expression of *S. aureus* Pol III-L holoenzyme was so high that it could

30   easily be visualized by Coomassie staining of a SDS polyacrylamide gel of whole cells (Figure 2A). The expressed protein migrated in the SDS polyacrylamide gel in a position expected for a 165 kDa polypeptide. In this procedure, it is important that

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cells are induced at 15°C, as induction at 37°C produces a truncated version of Pol III-L holoenzyme, of approximately 130 kDa.

          Cells were collected by centrifugation at 5°C. Cells (12 g wet weight) were stored at -70°C. The following steps were performed at 4°C. Cells were thawed
5   and lysed in cell lysis buffer as described (final volume = 50 ml) and were passed through a French Press (Amico) at a minimum of 20,000 psi. PMSF (2 mM) was added to the lysate as the lysate was collected from the French Press. DNA was removed and the lysate was clarified by centrifugation. The supernatent was dialyzed for 1 h against Buffer A containing 50 mM NaCl. The final conductivity was
10   equivalent to 190 mM NaCl. Supernatent (24 ml, 208 mg) was diluted to 50 ml using Buffer A to bring the conductivity to 96 mM $MgCl_2$, and then was loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was eluted with a 160 ml linear gradient of Buffer A from 50 mM NaCl to 500 mM NaCl. Seventy five fractions (1.3 ml each) were collected (see Figure 2B). Aliquots
15   were analyzed for their ability to synthesize DNA, and 20 μl of each fraction was analyzed by Coomassie staining of an SDS polyacrylamide gel. Based on the DNA synthetic capability, and the correct size band in the gel, fractions 56-65 containing Pol III-L holoenzyme were pooled (22 ml, 31 mg). The pooled fractions were dialyzed overnight at 4°C against 50 mM phosphate (pH 7.6), 5 mM DTT, 0.1 mM
20   EDTA, 2 mM PMSF, and 20 % glycerol (P-cell buffer). The dialyzed pool was loaded onto a 4.5 ml phosphocellulose column equilibrated in P-cell buffer, and then eluted with a 25 ml linear gradient of P-cell buffer from 0 M NaCl to 0.5 M NaCl. Fractions of 1 ml were collected and analyzed in a SDS polyacrylamide gel stained with Coomassie Blue (see Figure 2C). Fractions 20-36 contained the majority of the
25   Pol III-large at a purity of greater than 90 % (5 mg).

Example 7 - *S. aureus* Pol III-L is Not Processive on its Own

          The Pol III-L holoenzyme purifies from *B. subtilis* as a single subunit
30   without accessory factors (Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference). Hence, it seemed possible that it may be a Type I

replicase (e.g., like T5 polymerase) and, thus, be capable of extending a single primer full length around a long singly primed template. To perform this experiment, a template M13mp18 ssDNA primed with a single DNA oligonucleotide either in the presence or absence of SSB was used. DNA products were analyzed in a neutral
5   agarose gel which resolved products by size. The results showed that Pol III-L holoenzyme was incapable of extending the primer around the DNA (to form a completed duplex circle referred to as replicative form II (RFII)) whether SSB was present or not. This experiment has been repeated using more enzyme and longer times, but no full length RFII products are produced. Hence, Pol III-L would appear
10   not to follow the paradigm of the T5 system (Type I replicase) in which the polymerase is efficient in synthesis in the absence of any other protein(s).

Example 8 - Cloning and Purification of *S. aureus* Beta Subunit

15   The sequence of an *S. aureus* homolog of the *E. coli* dnaN gene (encoding the beta subunit) was obtained in a study in which the large recF region of DNA was sequenced (Alonso, et al., "Nucleotide Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 246:680-686 (1995), Alonso, et al., "Nucleotide
20   Sequence of the recF Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 248:635-636 (1995), which are hereby incorporated by reference). Sequence alignment of the *S. aureus* beta and *E. coli* beta show approximately 30% identity. Overall this level of homology is low and makes it uncertain that *S. aureus* beta will
25   have the same shape and function as the *E. coli* beta subunit.

            To obtain *S. aureus* beta protein, the dnaN gene was isolated and precisely cloned into a pET vector for expression in *E. coli*. *S. aureus* genomic DNA was used as template to amplify the homolog of the dnaN gene (encoding the putative beta). The upstream and downstream primers were designed to isolate the dnaN gene
30   by PCR amplification from genomic DNA. Primers were: upstream 5'-CGACTGGAAGGAGTTTTAACATATGATGGAATTCAC-3' (SEQ. ID. No. 19); the NdeI site used for cloning into pET16b is underlined. The downstream primer

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was 5'-TTATAT<u>GGATCC</u>TTAGTAAGTTCTGATTGG-3' (SEQ. ID. No. 20); where
the BamHI site used for cloning into pET16b (Novagen) is underlined. The NdeI and
BamHI sites were used for directional cloning into pET16 (Figure 3). Amplification
was performed using 500 ng genomic DNA, 0.5 mM dNTPs, 1 μM of each primer,

5   1mM MgSO$_4$, 2 units vent DNA polymerase in 100 ul of vent buffer. Forty cycles
were performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.;
72°C, 1 min. 10s. The 1167 bp product was digested with NdeI and BamHI and
purified in a 0.7 % agarose gel. The pure digested fragment was ligated into the
pET16b vector which had been digested with NdeI and BamHI and gel purified in a

10   0.7% agarose gel. Ligated products were transformed into *E. coli* competent SURE II
cells (Stratagene) and colonies were screened for the correct chimera by examining
minipreps for proper length and correct digestion products using NdeI and BamHI.

24 L of of BL21(DE3)pETbeta cells were grown in LB containing 50
μg/ml ampicillin at 37 °C to an O.D. of 0.7, and, then, the temperature was lowered to

15   15°C. IPTG was added to a concentration of 2 mM and after a further 18 h at 15°C
to induce expression of *S. aureus* beta (Figure 4A). It is interesting to note that the
beta subunit, when induced at 37°C, was completely insoluble. However, induction
of cells at 15°C provided strong expression of beta and, upon cell lysis, over 50% of
the beta was present in the soluble fraction.

20   Cells were harvested by centrifugation (44 g wet weight) and stored at
-70°C. The following steps were performed at 4°C. Cells (44 g wet weight) were
thawed and resuspended in 45 ml 1X binding buffer (5 mM imidizole, 0.5 M NaCl, 20
mM Tris HCl (final pH 7.5)) using a dounce homogenizer. Cells were lysed using a
French Pressure cell (Aminco) at 20,000 psi, and then 4.5 ml of 10 % polyamine P

25   (Sigma) was added. Cell debris and DNA was removed by centrifugation at 13,000
rpm for 30 min. at 4°C. The pET16beta vector places a 20 residue leader containing
10 histidine residues at the N-terminus of beta. Hence, upon lysing the cells, the
S. aureus beta was greatly purified by chromatography on a nickel chelate resin
(Figure 4B). The supernatant (890 mg protein) was applied to a 10 ml HiTrap

30   Chelating Separose column (Pharmacia-LKB) equilibrated in binding buffer. The
column was washed with binding buffer, then eluted with a 100 ml linear gradient of

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60 mM imidazole to 1 M imidazole in binding buffer. Fractions of 1.35 ml were

collected. Fractions were analyzed for the presence of beta in an SDS polyacrylamide

gel stained with Coomassie Blue. Fractions 28-52, containing most of the beta

subunit, were pooled (35 ml, 82 mg). Remaining contaminating protein was removed

5   by chromatography on MonoQ. The *S. aureus* beta becomes insoluble as the ionic

strength is lowered, and, thus, the pool of beta was dialyzed overnight against Buffer

A containing 400 mM NaCl. The dialyzed pool became slightly turbid indicating it

was at its solubility limit at these concentrations of protein and NaCl. The insoluble

material was removed by centrifugation (64 mg remaining) and, then, diluted 2-fold

10   with Buffer A to bring the conductivity to 256. The protein was then applied to an 8

ml MonoQ column equilibrated in Buffer A plus 250 mM NaCl and then eluted with a

100 ml linear gradient of Buffer A from 0.25M NaCl to 0.75 M NaCl; fractions of

1.25 ml were collected (Figure 4C). Under these conditions, approximately 27 mg of

the beta flowed through the column and the remainder eluted in fractions 1-18

15   (24 mg).

Example 9 -   **The *S. aureus* Beta Subunit Protein Stimulates *S. aureus* Pol III-**
                     L and *E. coli* Core.

20   The experiment of Figure 5A, tests the ability of *S. aureus* beta to

stimulate *S. aureus* Pol III-L on a linear polydA-oligodT template. Reactions are also

performed with *E. coli* beta and Pol III core. The linear template was polydA of

average length of 4500 nucleotides primed with a 30mer oligonucleotide of T

residues. The first two lanes show the activity of Pol III-L either without (lane 1) or

25   with *S. aureus* beta (lane 2). The result shows that the *S. aureus* beta stimulates Pol

III-L approximately 5-6 fold. Lanes 5 and 6 show the corresponding experiment

using *E. coli* core with (lane 6) or without (lane 5) *E. coli* beta. The core is stimulated

over 10-fold by the beta subunit under the conditions used.

           Although gram positive and gram negative cells diverged from one

30   another long ago and components of one polymerase machinery would not be

expected to be interchangable, it was decided to test the activity of the *S. aureus* beta

with *E. coli* Pol III core. Lanes 3 and 4 shows that the *S. aureus* beta also stimulates

E. coli core about 5-fold. This result can be explained by an interaction between the

clamp and the polymerase that has been conserved during the evolutionary divergence of gram positive and gram negative cells. A chemical inhibitor that would disrupt this interaction would be predicted to have a broad spectrum of antibiotic activity, shutting down replication in gram negative and gram positive cells alike. This assay, and others based on this interaction, can be devised to screen chemicals for such inhibition. Further, since all the proteins in this assay are highly overexpressed through recombinant techniques, sufficient quantities of the protein reagents can be obtained for screening hundreds of thousands of compounds.

In summary, the results show that *S. aureus* beta, produced in *E. coli*, is indeed an active protein (i.e. it stimulates polymerase activity). Furthermore, the results shows that Pol III-L functions with a second protein (i.e. *S. aureus* beta). Before this experiment, there was no assurance that Pol III-L, which is significantly different in structure from *E. coli* alpha, would function with another protein. For example, unlike *E. coli* alpha, which copurifies with several accessory proteins, Pol III-L purified from *B. subtilis* purifies as a single protein with no other subunits attached (Barnes, et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference). Finally, if one were to assume that *S. aureus* beta would function with a polymerase, the logical candidate would have been the product of the dnaE gene instead of PolC (Pol III-L) since the dnaE product is more homologous to *E. coli* alpha subunit than Pol III-L.

Example 10 - The *S. aureus* Beta Subunit Behaves as a Circular Sliding Clamp

The ability of *S. aureus* beta to stimulate Pol III-L could be explained by formation of a 2-protein complex between Pol III-L and beta to form a processive replicase similar to the Type II class (e.g. T7 type). Alternatively, the *S. aureus* replicase is organized as the Type III replicase which operates with a circular sliding clamp and a clamp loader. In this case, the *S. aureus* beta would be a circular protein and would require a clamp loading apparatus to load it onto DNA. The ability of the beta subunit to stimulate Pol III-L in Figure 5A could be explained by the fact that the polydA-oligodT template is a linear DNA and a circular protein could thread itself

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onto the DNA over an end. Such "end threading" has been observed with PCNA and explains its ability to stimulate DNA polymerase delta in the absence of the RFC clamp loader (Burgers, et al., "ATP-Independent Loading of the Proliferating Cell Nuclear Antigen Requires DNA Ends," J. Biol. Chem., 268:19923-19926 (1993),

5   which is hereby incorporated by reference).

To distinguish between these possibilities, *S. aureus* beta was examined for ability to stimulate Pol III-L on a circular primed template. In Figure 5B, assays were performed using circular M13mp18 ssDNA coated with *E. coli* SSB and primed with a single oligonucleotide to test the activity of beta on

10   circular DNA. Lane 1 shows the extent of DNA synthesis using Pol III-L alone. In lane 2, Pol III-L was supplemented with *S. aureus* beta. The *S. aureus* beta did not stimulate the activity of Pol III-L on this circular DNA (nor in the absence of SSB). Inability of *S. aureus* beta to stimulate Pol III-L is supported by the results of Figure 6, lane 1 that analyzes the product of Pol III-L action on the circular DNA in

15   an agarose gel in the presence of *S. aureus* beta. In summary, these results show that *S. aureus* beta only stimulates Pol III-L on linear DNA, not circular DNA. Hence, the *S. aureus* beta subunit behaves as a circular protein.

Lane 3 shows the result of adding both *S. aureus* beta and *E. coli* gamma complex to Pol III-L. Again, no stimulation was observed (compare with lane

20   1). This result indicates that the functional contacts between the clamp and clamp loader were not conserved during evolution of gram positive and gram negative cells.

Controls for these reactions on circular DNA are shown for the *E. coli* system in Lanes 4-6. Addition of only beta to *E. coli* Pol III core did not result in stimulating the polymerase (compare lanes 4 and 5). However, when gamma

25   complex was included with beta and core, a large stimulation of synthesis was observed (lane 6). In summary, stimulation of synthesis is only observed when both beta and gamma complex were present, consistent with inability of the circular beta ring to assemble onto circular DNA by itself.

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Example 11 -   Pol III-L Functions as a Pol III-Type Replicase with Beta and Gamma Complex to Become Processive

Next, it was determined whether *S. aureus* Pol III-L requires two

5   components (beta and gamma complex) to extend a primer full length around a circular primed template. In Figure 6, a template circular M13mp18 ssDNA primed with a single DNA oligonucleotide was used. DNA products were analyzed in a neutral agarose gel which resolves starting materials (labeled ssDNA in Figure 6) from completed duplex circles (labelled RFII for replicative form II). The first two

10   lanes show, as demonstrated in other examples, that Pol III-L is incapable of extending the primer around the circular DNA in the presence of only *S. aureus* beta. In lane 4 of Figure 6, *E. coli* gamma complex and beta subunit were mixed with *S. aureus* Pol III-L in the assay containing singly primed M13mp18 ssDNA coated with SSB. If the beta clamp, assembled on DNA by gamma complex, provides

15   processivity to *S. aureus* Pol III-L, the ssDNA circle should be converted into a fully duplex circle (RFII) which would be visible in an agarose gel analysis. The results of the experiment showed that the *E. coli* beta and gamma complex did indeed provide Pol III-L with ability to fully extend the primer around the circular DNA to form the RFII (lane 4). The negative control using only *E. coli* gamma complex and beta is

20   shown in lane 3. For comparison, lane 6 shows the result of mixing the three components of the *E. coli* system (Pol III core, beta and gamma complex). This reaction gives almost exclusively full length RFII product. The qualitatively different product profile that Pol III-L gives in the agarose gel analysis compared to *E. coli* Pol III core with beta and gamma complex shows that the products observed using Pol III-

25   L is not due to a contaminant of *E. coli* Pol III core in the *S. aureus* Pol III-L preparation (compare lanes 4 and 6).

It is generally thought that the polymerase of one system is specific for its SSB. However, these reactions are performed on ssDNA coated with the *E. coli* SSB protein. Hence, the *S. aureus* Pol III-L appears capable of utilizing *E. coli* SSB

30   and the *E. coli* beta. It would appear that the only component that is not interchangeable between the gram positive and gram negative systems is the gamma complex.

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Thus, the *S. aureus* Pol III-L functions as a Pol III type replicase with the *E. coli* beta clamp assembled onto DNA by gamma complex.

Example 12 -   **Purification of Two DNA Polymerase III-Type Enzymes From *S.* aureus Cells**

The MonoQ resin by Pharmacia has very high resolution which would resolve the three DNA polymerases of *S. aureus*. Hence, *S. aureus* cells were lysed, DNA was removed from the lysate, and the clarified lysate was applied onto a MonoQ column. The details of this procedure are: 300 L of *S. aureus* (strain 4220 (a gift of Dr. Pat Schlievert, University of Minnisota)) was grown in 2X LB media at 37°C to an OD of approximately 1.5 and then were collected by centrifugation. Approximately 2 kg of wet cell paste was obtained and stored at -70°C. 122 g of cell paste was thawed and resuspended in 192 ml of cell lysis buffer followed by passage through a French Press cell (Aminco) at 40,000 psi. The resultant lysate was clarified by high speed centrifugation (1.3 g protein in 120 ml). A 20 ml aliquot of the supernatant was dialyzed 2 h against 2 L of buffer A containing 50 mM NaCl. The dialyzed material (148 mg, conductivity = 101 mM NaCl) was diluted 2-fold with Buffer A containing 50 mM NaCl and then loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was washed with Buffer A containing 50 mM NaCl, and then eluted with a 160 ml linear gradient of 0.05 M NaCl to 0.5 M NaCl in Buffer A. Fractions of 2.5 ml (64 total) were collected, followed by analysis in an SDS polyacrylamide gel and for their replication activity in assays using calf thymus DNA.

Three peaks of DNA polymerase activity were identified (Figure 7). Previous studies of cell extracts prepared from the gram positive organism, *Bacillus subtilis*, identified only two peaks of activity off a DEAE column (similar charged resin to MonoQ). The first peak was Pol II, and the second peak was a combination of DNA polymerases I and III. The DNA polymerases I and III were then separated on a subsequent phosphocellulose column. The middle peak in Figure 7 is much larger than the other two peaks, and, thus, it was decided to chromatograph this peak on a phosphocellulose column. The second peak of DNA synthetic activity was pooled (fractions 37-43; 28 mg in 14 ml) and dialyzed against 1.5 L P-cell buffer for

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2.5 h. Then, the sample (ionic strength equal to 99 mM NaCl) was applied to a 5 ml phosphocellulose column equilibrated in P-cell buffer. After washing the column in 10 ml P-cell buffer, the column was eluted with a 60 ml gradient of 0 - 0.5 M NaCl in P-cell buffer. 70 fractions were collected. Fractions were analyzed for DNA

5   synthesis using calf thymus DNA as template.

This column resolved the polymerase activity into two distinct peaks (Figure 7B). Hence, there appear to be four DNA polymerases in *Staphylococcus aureus*, which was designated here as peaks 1 (first peak off MonoQ), peak 2 (first peak off phosphocellulose), peak 3 (second peak of phosphocellulose), and peak 4

10   (last peak off Mono Q) (see Figure 7). Peak 4 was presumably Pol III-L, as it elutes from MonoQ in a similar position as the Pol III-L expressed in *E. coli* (compare Figure 7A with Figure 2).

To test which peak contained a Pol III-type of polymerase, an assay was used in which the *E. coli* gamma complex and beta support formation of full

15   length RFII product starting from *E. coli* SSB coated circular M13mp18 ssDNA primed with a single oligonucleotide. In Figure 8, both Peaks 1 and 2 are stimulated by the *E. coli* gamma complex and beta subunit, and, in fact, Peaks 2 and 3 are inhibited by these proteins (the quantitation is shown below the gel in the figure). Further, the product analysis in the agarose gel shows full length RFII duplex DNA

20   circles only for peaks 1 and 4. These results, combined with the NEM, pCMB and KCl characteristics in Table 1 below, suggests that there are two Pol III-type DNA polymerases in *S. aureus*, and that these are partially purified in peaks 1 and 4.

Next, it was determined which of these peaks of DNA polymerase activity correspond to DNA polymerases I, II, and III, and which peak is the

25   unidentified DNA polymerase. In the gram postive bacterium *B. subtilis*, Pol III is inhibited by pCMB, NEM, and 0.15 M NaCl, Pol II is inhibited by KCl, but not NEM or 0.15 M KCL, and Pol I is not inhibited by any of these treatments (Gass, et al., "Further Genetic and Enzymological Characterization of the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," J. Biol. Chem., 248:7688-7700 (1973), which

30   is hereby incorporated by reference). Hence, assays were performed in the presence or absence of pCMB, NEM, and 0.15 M KCl (see Table 1 below). Peak 3 clearly corresponded to Pol I, because it was not inhibited by NEM, pCMB, or 0.15 M NaCl.

Peak 2 correspond to Pol II, because it was not inhibited by NEM, but was inhibited

by pCMB and 0.15 M NaCl. Peaks 1 and 4 both had characteristics that mimic Pol

III; however, peak 4 elutes on MonoQ at a similar position as Pol III-L expressed in

E. coli (see Figure 2B). Hence, peak 4 is likely Pol III-L, and peak 1 is likely the

5   unknown polymerase.

<u>Table 1</u>

Expected Characteristics Polymerase	pCMB	NEM	0.15M KC1
Pol I	not inhibited*	not inhibited	not inhibited
Pol II	inhibited**	not inhibited	not inhibited
Pol III-L	inhibited	inhibited	not inhibited

10   *Not inhibited is defined as greater than 75% remaining activity
     ** Inhibited is defined as less than 40% remaining activity

Observed Characteristics			
Peak	PCMB	NEM	0.15M KCL assignment
peak1	inhibited	inhibited	new polymerase
Peak2	inhibited	not inhibited	Pol II
Peak3	not inhibited	not inhibited	Pol I
Peak4	inhibited	inhibited	Pol III-L

15   **Example 13 -   Demonstration That Peak 1 (Pol III-2) Functions as a Pol III-
                       Type Replicase With *E. coli* Beta Assembled on DNA by *E. coli*
                       Gamma Complex.**

                       It is interesting to note that the characteristics of peak 1 are similar to

20   those of a Pol III-type of DNA polymerase. To test whether peak 1 contained a Pol

     III type of polymerase, an assay in which the *E. coli* gamma complex and beta support

     formation of full length RFII product starting from *E. coli* SSB coated circular

     M13mp18 ssDNA primed with a single oligonucleotide was carried out. In Figure 8,

     both Peaks 1 and 2 are stimulated by the *E. coli* gamma complex and beta subunit,

25   and, in fact, Peaks 2 and 3 are inhibited by these proteins (the quantitation is shown

     below the gel in the Figure 8). Further, the product analysis in the agarose gel shows

     full length RFII duplex DNA circles only for peaks 1 and 4. These results, combined

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with the NEM, pCMB, and KCl characteristics in the Table above, suggests that there are two Pol III-type DNA polymerases in *S. aureus*, and that these are partially purified in peaks 1 and 4.

5   **Example 14** -   **Identification and Cloning of *S. aureus* dnaE**

This invention describes the finding of two DNA polymerases that function with a sliding clamp assembled onto DNA by a clamp loader. One of these DNA polymerases is likely Pol III-L, but the other has not been identified previously.

10   Presumably the chromatographic resins used in earlier studies did not have the resolving power to separate the enzyme from other polymerases. This would be compounded by the low activity of Pol III-2. To identify a gene encoding a second Pol III, the amino acid sequences of the Pol III alpha subunit of *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Haemophilis influenzae*, and *Helicobacter*

15   *pylori* were aligned using Clustal W (1.5). Two regions about 400 residues apart were conserved and primers were designed for the following amino acid sequences: upstream, LLFERFLNPERVSMP (SEQ. ID. No. 21) (corresponds in *E. coli* to residues 385-399); downstream KFAGYGFNKSHSAAY (SEQ. ID. No. 22) (corresponds in *E. coli* to residues 750-764). The following primers were designed to

20   these two peptide regions using codon preferences for *S. aureus*: upstream,

5' CTTCTTTTTGAAAGATTTCTAAATAAAGAACGTTATTCAATGCC 3' (SEQ. ID. No. 23); downstream,

5' ATAAGCTGCAGCATGACTTTTATTAAAACCATAACCTGCAAATTT 3' (SEQ. ID. No. 24). Amplification was performed using 2.5 units of Taq DNA

25   Polymerase (Gibco, BRL), 100 ng *S. aureus* genomic DNA, 1 mM of each of the four dNTPs, 1 μM of each primer, and 3 mM $MgCl_2$ in 100 μl of Taq buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 90 sec. The PCR product (approximately 1.1 kb) was electrophoresed in a 0.8 % agarose gel, and purified using a Geneclean III kit (Bio 101). The product was then divided

30   equally into ten separate aliquots, and used as a template for PCR reactions, according to the above protocol, to reamplify the fragment for sequencing. The final PCR product was purified using a Quiagen Quiaquick PCR Purification kit, quantitated via

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optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reactions.

5   Next, additional PCR primers were designed to obtain more sequence information 3' to the amplified section of the sequnce of dnaE. The upstream primer was: 5' AGTTAAAAATGCCATATTTTGACGTGTTTTAGTTCTAAT 3' (SEQ. ID. No. 25), and the downstream primer was, 5' CTTGCAAAAGCGGTTGCTAAAGATGTTGGACGAATTATGGGG 3' (SEQ. ID. No. 26).

10   These primers were used in a PCR reaction using 2.5 units of Taq DNA Polymerase (Gibco, BRL) with 100 ng. *S. aureus* genomic DNA as a template, 1mM dNTP's , 1 M of each prmer, 3 mM MgCl$_2$ in 100 l of Taq buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 2 min 30 seconds. The 1.6 Kb product was then divided into 5 aliquots, and used as a

15   template in a set of 5 PCR reactions, as described above, to amplify the product for sequencing. The products of these reactions were purified using a Qiagen Qiaquick PCR Purification kit, quantitated via optical density at 260 nm, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The sequence of this product yielded about 740 bp of new sequence 3' of the first sequence. As this gene

20   shows better homology to gram negative pol III subunit compared to gram positive Pol III-L, it will be designated the dnaE gene.

As this gene shows better homology to the gram negative Pol III α subunit compared to gram positive Pol III-L, it will be designated the dnaE gene.

25   **Example 15 -   Identification and Cloning of *S. aureus* dnaX**

The fact that the *S. aureus* beta stimulates Pol III-L and has a ring shape suggests that the gram postive replication machinery is of the three component type. This implies the presence of a clamp loader complex. This is not a simple

30   determination to make as the *B. subtilis* genome shows homologs to only two of the five subunits of the *E. coli* clamp loader (dnaX encoding gamma, and holB encoding delta prime). On the basis of the experiments in this application, which suggests that

there is a clamp loader, we now presume these two subunit homologues are part of the
clamp loader for the *S. aureus* beta.

As a start in obtaining the clamp loading apparatus, a strategy was
devised to obtain the gene encoding the tau/gamma subunit of *S. aureus*. In *E. coli*,
5   these two subunits are derived from the same gene. Tau is the full length product, and
gamma is about 2/3 the length of tau. Gamma is derived from the dnaX gene by an
efficient translational frameshift mechanism that after it occurs incorporates only one
unique C-terminal reside before encountering a stop codon. To identify the dnaX
gene of *S. aureus* by PCR analysis, the dnaX genes of *B. subtilis*, *E. coli*, and *H.*
10   *influenzae* were aligned. Upon comparison of the amino acid sequence encoded by
these *dnaX* genes, two areas of high homology were used to predict the amino acid
sequence of the *S. aureus* dnaX gene product. PCR primers were designed to these
sequences, and a PCR product of the expected size was indeed produced. DNA
primers were designed to two regions of high similarity for use in PCR that were
15   about 100 residues apart. The amino acid sequences of these regions were: upstream,
HAYLFSGPRG (SEQ. ID. No. 27) (corresponds to residues 39-48 of *E. coli*), and
downstream, ALLKTLEEPPE (SEQ. ID. No. 28) (corresponds to residues 138-148 of
E. coli). The DNA sequence of the PCR primers was based upon the codon usage of
S. aureus. The upstream 38mer was
20   5'-CGC<u>GGATCC</u>CATGCATATTTATTTTCAGGTCCAAGAGG-3' (SEQ. ID.
No. 29). The first 9 nucleotides contain a BamHI site and do not correspond to amino
acid codons; the 3' 29 nucleotides correspond to the amino acids: HAYLFSGPRG
(SEQ. ID. No. 30). The downstream 39 mer was
5'-CCG<u>GAATTC</u>TGGTGGTTCTTCTAATGTTTTTAATAATGC-3' (SEQ. ID.
25   No. 31). The EcoRI site is underlined and the 3' 33 nucleotides correspond to the
amino acid sequence: ALLKTLEEPPE (SEQ. ID. No. 32). The expected PCR
product, based on the alignment, is approximately 268 bp between the primer
sequences. Amplification was performed using 500 ng genomic DNA, 0.5 mM
dNTPs, 1 µM of each primer, 1 mM MgSO$_4$, 2 units vent DNA polymerase in 100 µl
30   of vent buffer. Forty cycles were performed using the following cycling scheme:
94°C, 1 min; 60°C, 1 min.; 72°C, 30s. The approximately 300 bp product was
digested with EcoRI and BamHI and purified in a 0.7 % agarose gel. The pure

digested fragment was ligated into pUC18 which had been digested with EcoRI and BamHI and gel purified in a 0.7 % agarose gel. Ligated products were transformed into *E. coli* competent DH5α cells (Stratagene), and colonies were screened for the correct chimera by examining minipreps for proper length and correct digestion

5   products using EcoRI and BamHI. The sequence of the insert was determined and was found to have high homology to the dnaX genes of several bacteria. This sequence was used to design circular PCR primers. Two new primers were designed for circular PCR based on this sequence.

A circular PCR product of approximately 1.6 kb was obtained from a

10   HincII digest of chromosomal DNA that was recircularized with ligase. This first circular PCR yielded most of the remaining dnaX gene. The rightward directed primer was 5'-TTT GTA AAG GCA TTA CGC AGG GGA CTA ATT CAG ATG TG-3' (SEQ. ID. No. 33); the sequence of the leftward primer was 5'-TAT GAC ATT CAT TAC AAG GTT CTC CAT CAG TGC-3' (SEQ. ID. No. 34).

15   Genomic DNA (3 μg) was digested with HincII, purified with phenol/chloroform extraction, ethanol precipitated and redissolved in 70 μl T.E. buffer. The genomic DNA was recircularized upon adding 4000 units T4 ligase (New England Biolabs) in a final volume of 100 μl T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each

20   dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 μl elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min., 55°C, 1 min., and 68°C, 2 min. The resulting PCR product was approximately 1.6 kb. The PCR product was purified from a 0.7 % agarose gel and sequenced directly. A stretch of approximately 750

25   nucleotides was obtained using the rightward primer used in the circular PCR reaction. To obtain the rest of the sequence, other sequencing primers were designed in succession based on the information of each new sequencing run.

This sequence, when spliced together with the previous 300 bp PCR sequence, contained the complete N-terminus of the gene product (stop codons are

30   present upstream) and possibly lacked only about 50 residues of the C-terminus. The amino terminal region of *E. coli* gamma/tau shares appears the most conserved region of the gene as this area shares homology with RFC subunit of the human clamp loader

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and with the gene 44 protein of the phage T4 clamp loader. An alignment of the N-terminal region of the *S. aureus* gamma/tau protein with that of *B. subtilis* and *E. coli* is shown in Figure 10. Among the highly conserved residues are the ATP binding site consensus sequence and the four cystine residues that form a Zn++ finger.

5   After obtaining 1 kb of sequence in the 5' region of dnaX, it was sought to determine the remaining 3' end of the gene. Circular PCR products of approximately 800bps, 600bps, and 1600bps were obtained from Apo I, or Nsi I or Ssp I digest of chromosomal DNA that were recircularized with ligase. The rightward directed primer was 5'-GAGCACTGATGAACTTAGAATTAGATATG-3' (SEQ. ID.

10   No. 35); the sequence of the leftward primer was 5'-GATACTCAGTATCTTTCTCAGATGTTTTATTC-3' (SEQ. ID. No. 36). Genomic DNA (3 g) was digested with, Apo I, or Nsi I or Ssp I, purified with phenol/chloroform extraction, ethanol precipitated, and redissolved in 70 l T.E. buffer. The genomic DNA was recircularized upon adding 4000 units of T4 ligase

15   (New England Biolabs) in a final volume of 100 l T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 l elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min.;

20   55°C, 1 min.; 68°C, 2 min. The PCR products were directly cloned into pCR II TOPO vector using the TOPO TA cloning kit (Invitrogen Corporation) for obtaining the rest of the C terminal vector using the TOPO TA cloning kit (Invitrogen Corporation) for obtaining the rest of the C terminal sequence of *S. aureus* dnaX. DNA sequencing was performed by the Rockefeller University sequencing facility.

25

Example 16 -   Identification and Cloning of *S. aureus* dnaB

            In *E. coli*, the DnaB helicase assembles with the DNA polymerase III holoenzyme to form a replisome assembly. The DnaB helicase also interacts directly

30   with the primase to complete the machinery needed to duplicate a double helix. As a first step in studying how the *S. aureus* helicase acts with the replicase and primase, *S. aureus* was examined for presence of a dnaB gene.

The amino acid sequences of the DnaB helicase of *Escherichia coli*, *Salmonella typhimurium*, *Haemophilis influenzae*, and *Helicobacter pylori* were aligned using Clustal W (1.5). Two regions about 200 residues apart showed good homology. These peptide sequences were: upstream, DLIIVAARPSMGKT (SEQ.

5   ID. No. 37) (corresponds to residues 225-238 of *E. coli* DnaB), and downstream, EIIIGKQRNGPIGTV (SEQ. ID. No. 38) (corresponds to residues 435-449 of *E. coli*). The following primers were designed from regions which contained conserved sequences using codon preferences for *S. aureus*: The upstream primer was 5' GACCTTATAATTGTAGCTGCACGTCC TTCTAT GGGAAAAAC 3' (SEQ. ID.

10   No. 39); the dowstream primer was 5' AACATTATTAAGTCAGCATCTTGT TCTATTGATCCAGATTCAACGAAG 3' (SEQ. ID. No. 40). A PCR reaction was carried out using 2.5 units of Taq DNA Polymerase (Gibco, BRL) with 100 ng. *S. aureus* genomic DNA as template, 1 mM dNTP's, 1μM of each primer, 3 mM MgCl₂ in 100 μl of Taq buffer. Thirty-five cycles

15   of the following scheme were repeated: 94°C, 1 min.; 55°C, 1 min.; and 72°C, 1 min. Two PCR products were produced, one was about 1.1 kb, and another was 0.6 kb. The smaller one was the size expected. The 0.6 kb product was gel purified and used as a template for a second round of PCR as follows. The 0.6 kb PCR product was purified from a 0.8% agarose gel using a Geneclean III kit (Bio 101) and then divided

20   equally into five separate aliquots, as a template for PCR reactions. The final PCR product was purified using a Quiagen Quiaquick PCR Purification kit, quantitated via optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reaction. The amino acid sequence was determined by translation of the

25   DNA sequence in all three reading frames, and selecting the longest open reading frame. The PCR product contained an open reading frame over its entire length. The predicted amino acid sequence shares homology to the amino acid sequences encoded by dnaB gene of other organisms.

Additional sequence information was determined using the circular

30   PCR technique. Briefly, *S. aureus* genomic DNA was digested with various endonucleases, then religated with T4 DNA ligase to form circular templates. To perform PCR, two primers were designed from the initial sequence. The first primer,

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5' GATTTGTAGTTCTGGTAATGTTGACTCAAACCGCTTAAGAACCGG 3'

(SEQ ID. No. 41), matches the coding strand; the second primer, 5'

ATACGTGTGGTTAACTGATCAGCAACCCATCTCTAGTGAGAAAATACC 3'

(SEQ ID. No. 42), matches the sequence of the complementary strand. These two

5   primers are directed outwards from a central point, and allow determination of new

sequence information up to the ligated endonuclease site. A PCR product of

approximately 900 bases in length was produced using the above primers and

template derived from the ligation of *S. aureus* genomic DNA which had been cut

with the restriction endonuclease Apo I. This PCR product was electrophoresed in a

10   0.8% agarose gel, eluted with a Qiagen gel elution kit, divided into five separate

aliquots, and used as a template for reamplification by PCR using the same primers as

described above. The final product was electrophoresed in an 0.8% agarose gel,

visualized via staining with ethidium bromide under ultraviolet light, and excised

from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm for 15

15   minutes. The supernatant was extracted with phenol/chloroform to remove ethidium

bromide, and was then cleaned using a Qiagen PCR purification kit. The material was

then quantitated from its optical density at 260 nm and sequenced by the Protein/DNA

Technology Center at the Rockefeller University.

The nucleotide sequence contained an open reading frame over its

20   length, up to a sequence which corresponded to the consensus sequence of a cleavage

site of the enzyme Apo I. Following this point, a second open reading frame encoded

a different reading frame up to the end of the product. The inital sequence

information was found to match the inital sequence and to extend it yet further

towards the C-terminus of the protein. The second reading frame was found to end in

25   a sequence which matched the 5'-terminus of the previously determined sequence and,

thus, represents an extension of the sequence towards the N-terminus of the protein.

Additional sequence information was obtained using the above primers

and a template generated using *S. aureus* genomic DNA circularized via ligation with

T4 ligase following digestion with Cla I. The PCR product was generated using 35

30   cycles of the following program: denaturation at 94°C for 1 min.; annealing at 55°C

for 1 min.; and extension at 68°C for 3 minutes and 30 s. The PCR products were

electrophoresed in a 0.8% agarose gel, eluted with a Qiagen gel elution kit, divided

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into five separate aliquots, and used as a template reamplification via PCR with the same primers described above. The final product was electrophoresed in an 0.8% agarose gel, visualized via staining with ethidium bromide under ultraviolet light, and excised from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm

5   for 15 min. The supernatant was cleaned using a Qiagen PCR purification kit. The material was then quantitated via optical density at 260 nm and sequenced by the Protein/DNA Technology Center at Rockefeller University. The open reading frames continued past 500 bases. Therefore, the following additional sequencing primers were designed from the sequence to obtain further information:

10   5' CGTTTTAATGCATGCTTAGAAACGATATCAG 3' (SEQ. ID No. 43) and,
5' CATTGCTAAGCAACGTTACGGTCCAACAGGC 3' (SEQ. ID No. 44).

The N-terminal and C-terminal nucleotide sequence extensions generated using this circular PCR product completed the 5' region of the gene (encoding the N-terminus of DnaB); however, a stop codon was not reached in the 3'

15   region and, thus, a small amount of sequence is still needed to complete this gene.

The alignment of the *S. aureus* dnaB with *E. coli* dnaB and the dnaB genes of *B. subtilis* and *S. typhimurium* is shown in Figure 11.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations

20   can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1.   An isolated DNA molecule corresponding to dnaE from a Gram positive bacterium.

5

2.   An isolated DNA molecule according to claim 1, wherein the Gram positive bacterium is a *Staphlococcus*.

3.   An isolated DNA molecule according to claim 2, wherein the

10   Gram positive bacterium is *Staphlococcus aureus*.

4.   An isolated DNA molecule according to claim 3, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 2.

15   5.   An isolated DNA molecule according to claim 3, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 1 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 1.

6.   An expression system containing the DNA molecule according

20   to claim 1.

7.   An expression system according to claim 6, wherein the DNA molecule is in proper sense orientation and correct reading frame.

25   8.   A host cell transformed with the DNA molecule according to claim 1.

9.   A host cell according to claim 8, wherein the DNA molecule is in an expression system.

30

10.   An isolated dnaE protein from a Gram positive bacterium.

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11.   An isolated protein according to claim 10, wherein the Gram positive bacterium is a *Staphlococcus*.

12.   An isolated protein according to claim 11, wherein the Gram positive bacterium is *Staphlococcus aureus*.

13.   An isolated protein according to claim 12, wherein said protein has an amino acid sequence of SEQ. ID. No. 2.

14.   An isolated DNA molecule corresponding to dnaX from a Gram positive bacterium.

15.   An isolated DNA molecule according to claim 14, wherein the Gram positive bacterium is a *Staphlococcus*.

16.   An isolated DNA molecule according to claim 15, wherein the Gram positive bacterium is *Staphlococcus aureus*.

17.   An isolated DNA molecule according to claim 16, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 4.

18.   An isolated DNA molecule according to claim 16, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 3 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 3.

19.   An expression system containing the DNA molecule according to claim 16.

20.   An expression system according to claim 19, wherein the DNA molecule is in proper sense orientation and correct reading frame.

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21.   A host cell transformed with the DNA molecule according to claim 16.

22.   A host cell according to claim 21, wherein the DNA molecule is in an expression system.

23.   An isolated dnaX protein from a Gram positive bacterium.

24.   An isolated protein according to claim 23, wherein the Gram positive bacterium is a *Staphlococcus*.

25.   An isolated protein according to claim 24, wherein the Gram positive bacterium is *Staphlococcus aureus*.

26.   An isolated protein according to claim 25, wherein said protein has an amino acid sequence of SEQ. ID. No. 4.

27.   An isolated DNA molecule corresponding to dnaB from a Gram positive bacterium.

28.   An isolated DNA molecule according to claim 27, wherein the Gram positive bacterium is a *Staphlococcus*.

29.   An isolated DNA molecule according to claim 28, wherein the Gram positive bacterium is *Staphlococcus aureus*.

30.   An isolated DNA molecule according to claim 29, wherein said DNA molecule encodes a protein having an amino acid sequence of SEQ. ID. No. 6.

31.   An isolated DNA molecule according to claim 29, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 5 or hybridizes under stringent conditions to a nucleotide sequence of SEQ. ID. No. 5.

32.   An expression system containing the DNA molecule according
to claim 27.

5   33.   An expression system according to claim 32, wherein the DNA
molecule is in proper sense orientation and correct reading frame.

           34.   A host cell transformed with the DNA molecule according to
   claim 27.

10

           35.   A host cell according to claim 34, wherein the DNA molecule
is in an expression system.

           36.   An isolated dnaB protein from a Gram positive bacterium.

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           37.   An isolated protein according to claim 36, wherein the Gram
positive bacterium is a *Staphlococcus*.

           38.   An isolated protein according to claim 37, wherein the Gram
20   positive bacterium is *Staphlococcus aureus*.

           39.   An isolated protein according to claim 38, wherein the protein
has an amino acid sequence of SEQ. ID. No. 6.

25   40.   A method of identifying compounds which inhibit the activity
of a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:
                   forming a reaction mixture comprising a primed DNA
molecule, a DNA polymerase from a Gram positive bacterium, a candidate
compound, and a dNTP;
30   subjecting the reaction mixture to conditions effective to
achieve nucleic acid polymerization in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

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41.   A method of identifying compounds which inhibit the ability of a beta subunit to stimulate a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:

forming a reaction mixture comprising a primed DNA

10   molecule, a DNA polymerase, a candidate compound, a beta subunit, and a dNTP;

subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound, wherein either or both the beta subunit and/or the DNA polymerase are derived from a Gram positive bacterium;

15   analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

20   42.   A method of identifying compounds which inhibit the ability of a beta subunit and a gamma complex to stimulate a Pol III 2 DNA polymerase or a Pol III L DNA polymerase comprising:

forming a reaction mixture comprising a primed DNA molecule, a DNA polymerase, a candidate compound, a beta subunit, a gamma

25   complex, and a dNTP;

subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound, wherein either or both of the beta subunit and/or the gamma complex or a subunit or combination of subunits thereof are derived from a Gram positive bacterium;

30   analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

identifying candidate compounds in reaction mixtures where there is an absence of nucleic acid polymerization extension products.

43.   A method of identifying compounds which inhibit the ability of a beta subunit and a DNA polymerase to interact physically comprising:

forming a reaction mixture comprising a beta subunit, a DNA polymerase, and a candidate compound;

subjecting the reaction mixture to conditions effective to permit the beta subunit and the DNA polymerase to interact in the absence of the candidate compound, wherein either or both the beta subunit and/or the DNA polymerase are derived from a Gram positive bacterium;

analyzing the reaction mixture for the presence or absence of interaction between the beta subunit and the DNA polymerase; and

identifying candidate compound in reaction mixtures where there is an absence of interaction between the beta subunit and the DNA polymerase.

44.   A method of identifying compounds which inhibit the ability of a beta subunit and a gamma complex or subunit(s) thereof to interact comprising:

forming a reaction mixture comprising a beta subunit, a gamma complex or subunit(s) thereof, and a candidate compound, wherein either or both of the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

subjecting the reaction mixture to conditions effective to permit the beta subunit and the gamma complex or subunit(s) thereof to interact in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of interaction between the beta subunit and the gamma complex or subunit(s) thereof; and

identifying the candidate compound in reaction mixtures where there is an absence of interaction between the beta subunit and the gamma complex or subunit(s) thereof.

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45.   A method of identifying compounds which inhibit the ability of
a gamma complex or subunit(s) thereof to assemble a beta subunit on a DNA
molecule comprising:

forming a reaction mixture comprising a circular primed DNA

5   molecule, a beta subunit, a gamma complex or subunit(s) thereof, an ATP, and a
candidate compound, wherein the beta subunit and/or the gamma complex or
subunit(s) thereof are derived from a Gram positive bacterium;

subjecting the reaction mixture to conditions effective to permit
the gamma complex or subunit(s) thereof to assemble the beta subunit on the DNA

10   molecule in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of
the beta subunit on the DNA molecule; and

identifying the candidate compound in reaction mixtures where
there is an absence of the beta subunit on the DNA molecule.

15

46.   A method of identifying compounds which inhibit the ability of
a gamma complex or subunit(s) thereof to disassemble a beta subunit from a DNA
molecule comprising:

forming a reaction mixture comprising a DNA molecule on to

20   which a beta subunit has been assembled, a gamma complex or subunit(s) thereof, and
a candidate compound, wherein the beta subunit and/or the gamma complex or
subunit(s) thereof are derived from a Gram positive bacterium;

subjecting the reaction mixture to conditions effective to permit
the gamma complex or subunit(s) thereof to disassemble the beta subunit from the

25   DNA molecule in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of
the beta subunit on the DNA molecule; and

identifying the candidate compound in reaction mixtures where
the beta subunit is on the DNA molecule.

30

47.   A method of identifying compounds which disassemble a beta
subunit from a DNA molecule comprising:

forming a reaction mixture comprising a circular DNA molecule on to which a beta subunit has been assembled, a gamma complex or subunit(s) thereof, and a candidate compound;

5   subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to disassemble the beta subunit from the DNA molecule in the absence of the candidate compound, wherein either or both the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

analyzing the reaction mixture for the presence or absence of
10   the beta subunit on the DNA molecule; and

identifying the candidate compound in reaction mixtures where the beta subunit is absent from the DNA molecule.

48.   A method of identifying compounds which inhibit dATP/ATP
15   binding activity of a gamma complex or subunit(s) thereof comprising:

forming a reaction mixture comprising a gamma complex or subunit(s) thereof, dATP/ATP in the presence or absence of a DNA molecule and/or a beta subunit, and a candidate compound, wherein either or both the beta subunit and/or the gamma complex or subunit(s) thereof are derived from a Gram positive
20   bacterium;

subjecting the reaction mixture to conditions effective to permit the gamma complex or subunit(s) thereof to interact with the dATP/ATP in the absence of the candidate compound;

analyzing the reaction mixture to determine whether or not the
25   dATP/ATP is bound to the gamma complex or subunit(s) thereof; and

identifying the candidate compound in reaction mixtures where the dATP/ATP is not bound to the gamma complex or subunit(s) thereof.

49.   A method of identifying compounds which inhibit dATP/ATP
30   binding activity of a gamma complex or subunit(s) thereof comprising:

forming a reaction mixture comprising a gamma complex or subunit(s) thereof, dATP/ATP in the presence or absence of a DNA molecule and/or a

beta subunit, and a candidate compound, wherein either or both the gamma complex
or subunit(s) thereof and/or the beta subunit are derived from a Gram positive
bacterium;

5   subjecting the reaction mixture to conditions effective to permit
the gamma complex or subunit(s) thereof to hydrolyze dATP/ATP in the absence of
the candidate compound, wherein either or both the gamma complex or subunit(s)
thereof and/or the beta subunit are derived from a Gram positive bacterium;

         analyzing the reaction mixture to determine whether or not
dATP/ATP is hydrolyzed; and

10   identifying the candidate compound in reaction mixtures where
dATP/ATP is not hydrolyzed.

         50.   A method of identifying compounds which inhibit a DNA
polymerase encoded by a dnaE gene or PolC gene comprising:

15   forming a reaction mixture comprising a primed DNA
molecule, a protein encoded by a dnaE gene or PolC gene from a Gram positive
bacterium, dNTP or modified dNTP, and a candidate compound;

         subjecting the reaction mixture to conditions effective to permit
nucleic acid polymerization and the resulting formation of an extension product in the

20   absence of the candidate compound;

         analyzing the reaction mixture for the presence or absence of
extension product; and

         identifying the candidate compound in reaction mixtures where
there is an absence of an extension product.

25

         51.   A method of identifying compounds which inhibit a DNA
polymerase encoded by a dnaE gene or PolC gene comprising:

         forming a reaction mixture comprising a primed, linear DNA
molecule, a protein encoded by a dnaE gene or PolC gene, dNTP or modified dNTP, a

30   beta subunit, and a candidate compound, wherein either or both the protein encoded
by the dnaE gene or PolC gene and/or the beta subunit are derived from a Gram
positive bacterium;

subjecting the reaction mixture to conditions effective to permit nucleic acid polymerization and the resulting formation of an extension product in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of

5   extension product; and

identifying the candidate compound in reaction mixtures where there is an absence of an extension product.

52.   A method of identifying compounds which inhibit a DNA

10   polymerase encoded by a dnaE gene or PolC gene comprising:

forming a reaction mixture comprising a primed, circular DNA molecule, a protein encoded by a dnaE gene or PolC gene, dNTP or modified dNTP, a beta subunit, a gamma complex or subunit thereof, and a candidate compound, wherein either or all of the protein encoded by the dnaE gene or PolC gene, the beta

15   subunit, and/or the gamma complex or subunit(s) thereof are derived from a Gram positive bacterium;

subjecting the reaction mixture to conditions effective to permit nucleic acid polymerization and the resulting formation of an extension product in the absence of the candidate compound;

20   analyzing the reaction mixture for the presence or absence of extension product; and

identifying the candidate compound in reaction mixtures where there is an absence of an extension product.

25   53.   A method of identifying compounds which inhibit a DNA polymerase encoded by a dnaE gene or PolC gene comprising:

forming a reaction mixture comprising a protein encoded by a dnaE gene or PolC gene, a beta subunit, and a candidate compound, wherein either or both the protein encoded by the dnaE gene or PolC gene and/or the beta subunit are

30   derived from a Gram positive bacterium;

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subjecting the reaction mixture to conditions effective to permit
the beta subunit to interact with the protein encoded by the dnaE gene or PolC gene in
the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of

5   interaction between the beta subunit and the protein encoded by the dnaE gene or
PolC gene; and

identifying the candidate compound in reaction mixtures where
there is an absence of interaction between the beta subunit and the protein encoded by
the dnaE gene or PolC gene.

10

54.   A method of identifying compounds which inhibit a DnaB
helicase comprising:

forming a reaction mixture comprising a DnaB helicase from a
Gram positive bacterium, a substrate DNA molecule having a duplex region, and a

15   nucleoside or deoxynucleoside triphosphate energy source;

subjecting the reaction mixture to conditions effective to
support helicase activity in the absence of the candidate compound;

analyzing the reaction mixture for conversion of the duplex
DNA molecule to a single stranded DNA molecule; and

20   identifying the candidate compound in reaction mixtures where
the duplex DNA molecule is not converted to a single stranded DNA molecule.

55.   A method of identifying compounds which inhibit nucleoside
or deoxynucleoside triphosphate activity of a DnaB helicase from a Gram positive

25   bacterium comprising:

forming a reaction mixture comprising a DnaB helicase, a
substrate DNA molecule having a duplex region, a nucleoside or deoxynucleoside
triphosphate energy source, and a candidate compound;

subjecting the reaction mixture to conditions effective to

30   support nucleoside or deoxynucleoside activity of DnaB in the absence of the
candidate compound;

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analyzing the reaction mixture for conversion of the nucleoside
or deoxynucleoside triphosphate to a nucleoside or deoxynucleoside diphosphate; and
identifying the candidate compound in reaction mixtures where
the nucleoside or deoxynucleoside triphosphate is not converted to the nucleoside or

5   deoxynucleoside diphosphate.

56.   A method of identifying compounds which inhibit primase
activity comprising:
forming a reaction mixture comprising a primase from a Gram

10   positive bacterium, a single stranded DNA molecule, and a candidate compound;
subjecting the reaction mixture to conditions effective to
support primase activity in the absence of the candidate compound;
analyzing the reaction mixture for the presence or absence of
primer formation; and

15   identifying the candidate compound in reaction mixtures where
no primers are formed.

57.   A method of identifying compounds which inhibit the ability of
a primase and a DnaB protein to interact comprising:

20   forming a reaction mixture comprising a primase, a DnaB
protein, and a candidate compound, wherein either or both the primase and/or DnaB
are derived from a Gram positive bacterium;
subjecting the reaction mixture to conditions effective to permit
the primase and the DnaB protein to interact in the absence of the candidate

25   compound;
analyzing the reaction mixture for the presence or absence of
interaction between the primase and the DnaB protein; and
identifying the candidate compound in reaction mixtures where
no interaction occurs between the primase and the DnaB protein.

30

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58.   A method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein from a Gram positive bacterium to interact comprising:

forming a reaction mixture comprising a DNA molecule, a DnaB protein from a Gram positive bacterium, and a candidate compound;

subjecting the reaction mixture to conditions effective to permit the DNA molecule and the DnaB protein to interact in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of interaction between the DNA molecule and the DnaB protein; and

identifying the candidate compound in reaction mixtures where no interaction occurs between the DNA molecule and the DnaB protein.

59.   A method according to any one of claims 40 to 58, wherein the Gram positive bacterium is a *Staphlococcus*.

60.   A method according to any one of claims 40 to 58, wherein the Gram positive bacterium is a *Staphlococcus aureus*.
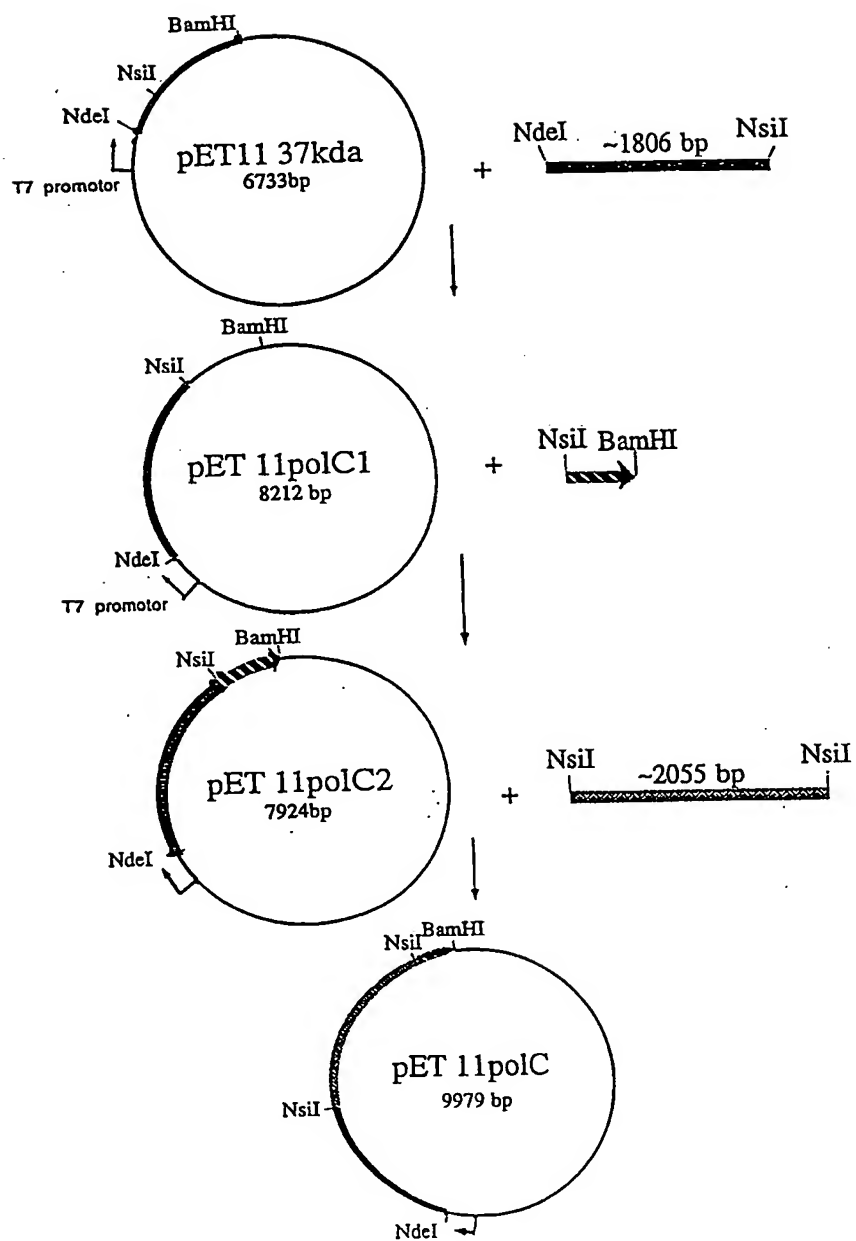
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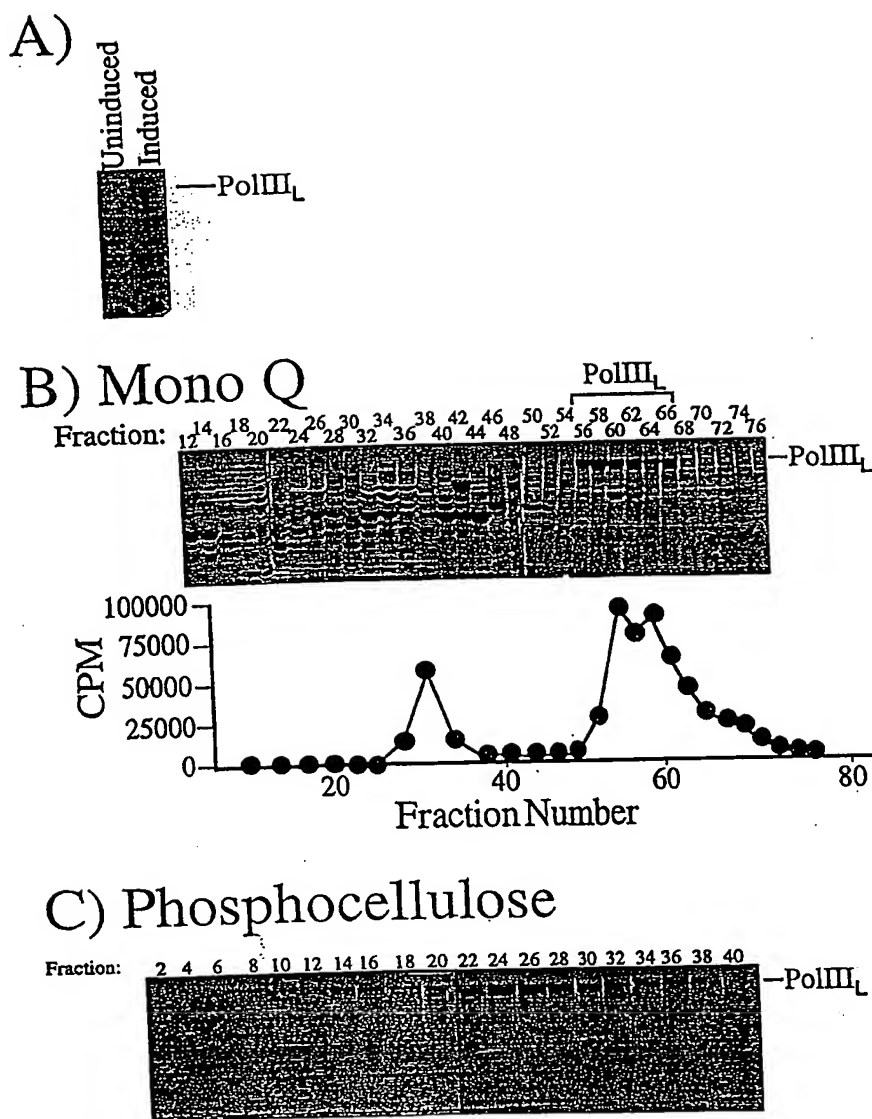
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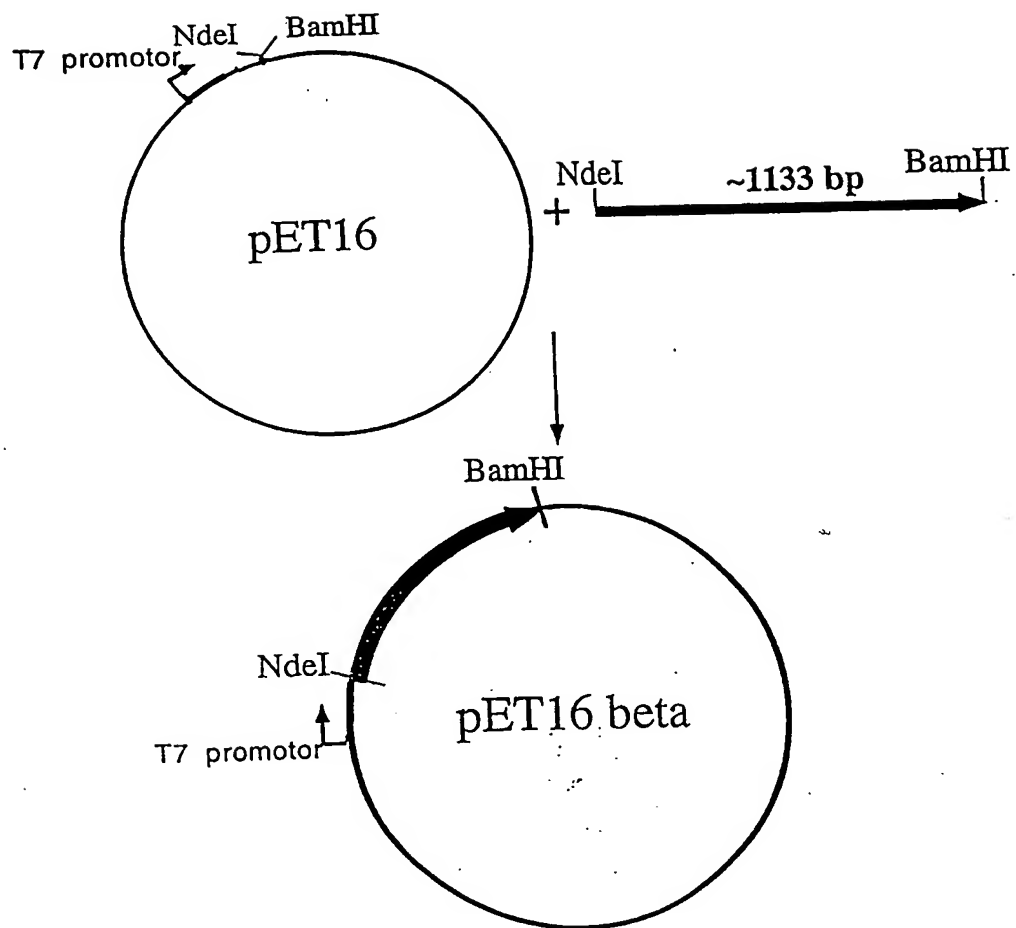
FIGURE 3

A) Induction
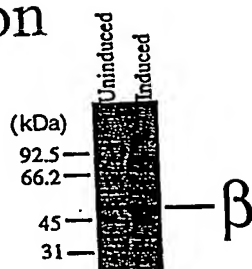
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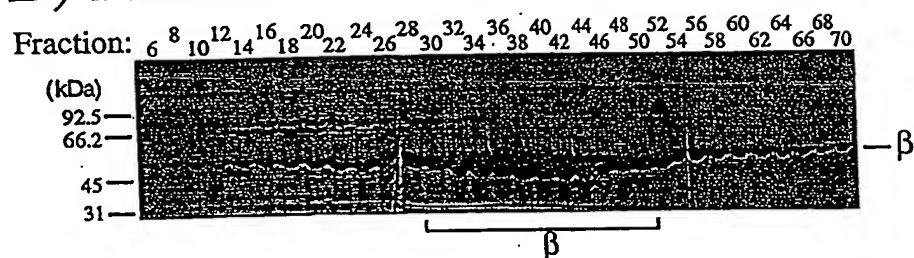
C) Mono Q

FIGURE 4

A) Linear DNA

	1	2	3	4	5	6
S. aur Pol III$_L$	+	+	–	–	–	–
S. aur. β	–	+	–	+	–	–
E. coli Pol III core	–	–	+	+	+	+
E. coli β	–	–	–	–	–	+

B) Circular DNA

	1	2	3	4	5	6
S. aur Pol III$_L$	+	+	+	–	–	–
S. aur. β	–	+	+	–	–	–
E. coli Pol III core	–	–	–	+	+	+
E. coli β	–	–	–	–	+	+
E. coli γ complex	–	–	+	–	–	+
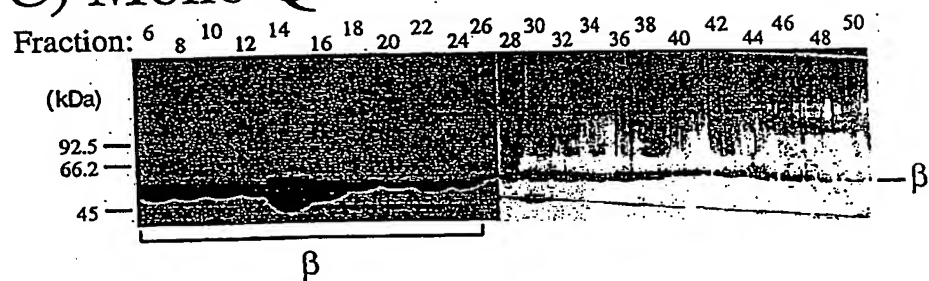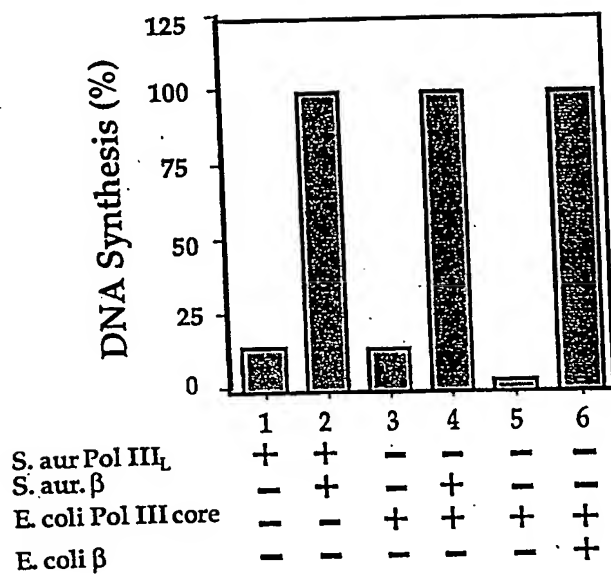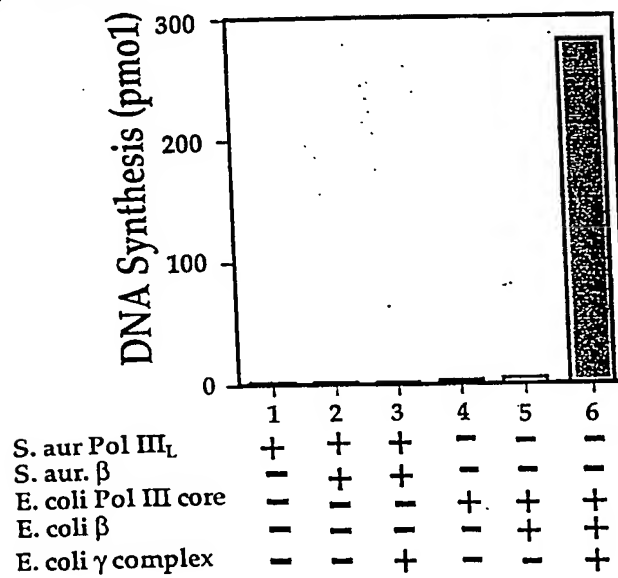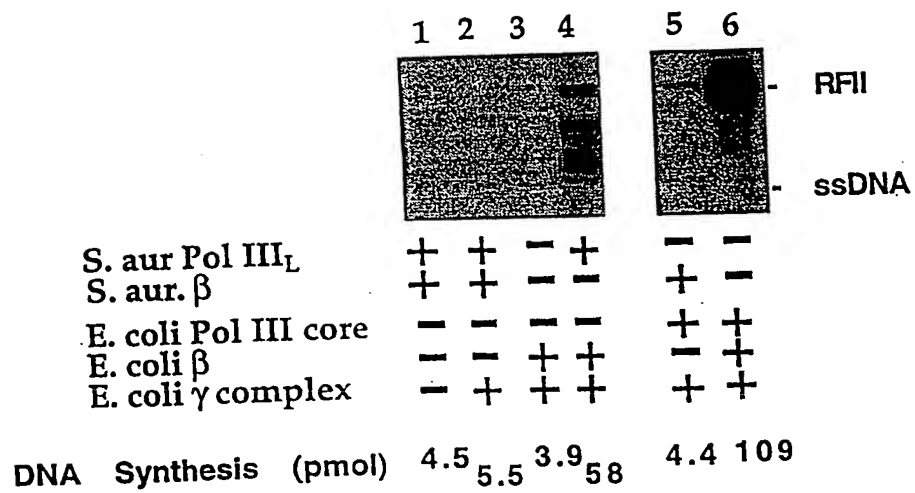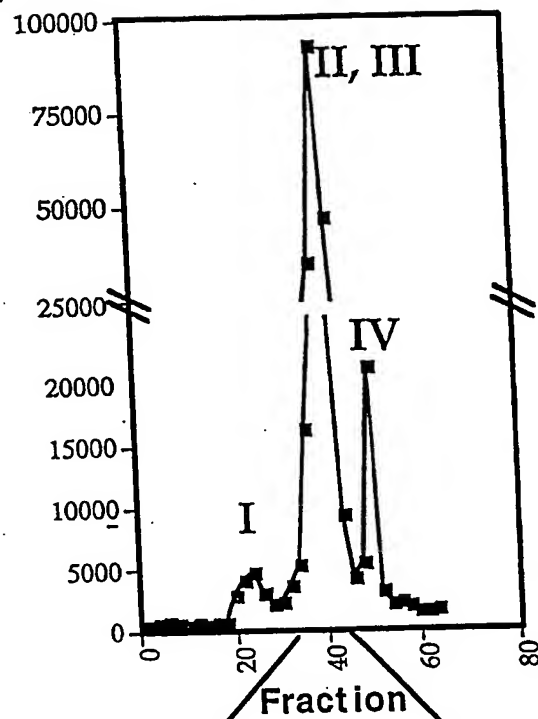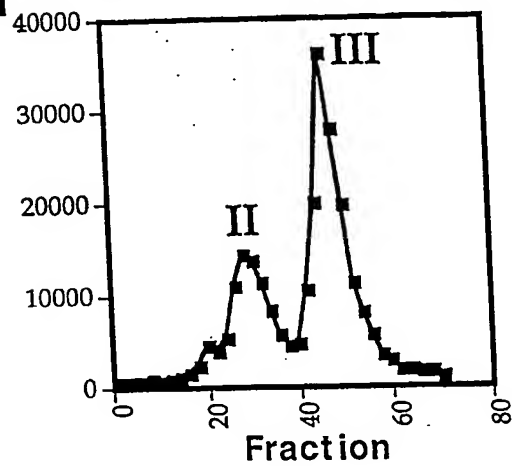
FIGURE 5

FIGURE 6

FIGURE 7

A) Agarose Gel
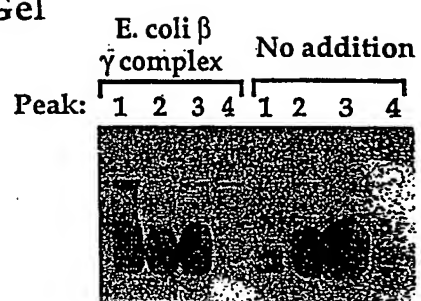
B) DNA Synthesis

	DNA Synthesis (pmol) PEAK			
Addition	Peak 1	Peak 2	Peak 3	Peak 4
None	22.7	70.6	146.1	4.7
E. coli β, γ complex	72.9	61.2	71.4	25.9

FIGURE 8

```
S. aureus   KIWRATCIWNCDFRSSACKAVAKDVGRIMGFPDEVTLNEISSLIPHKLGITLDEAYQID-D
E. coli     MYGRDAVSQIITFGTMAAKAVIRDVGRVLGHPYGFVDRISKLIPPDPGMTLAKAFEAEPQ
Sal. typ    MYGRDAVSQIITFGTMAAKAVIRDVGRVLGHPYGFVDRISKLVPPDPGMTLAKAFEAEPQ
                      *     *  ***  ...  *         ** * *  .**  *    .

S. aureus   FKKFVHRNRHQRWFSICKKLEGLPRHTSTHAAGIIINDHPLYEYAPLTKGDTG--LLTQ
E. coli     LPEIYEADEEVKALIDMARKLEGVTRNAGKHAGGVVIAPTKITDFAPLYCDEEGKHPVTQ
Sal. typ    LPEIYEADEEVRALIDMARKLEGVTRNAGKHAGGVVIAPTKITDFAPLYCDEEGKHPVTQ
             .  *  .   .      ****   .     ..* **.   **  ***        **

S. aureus   WIMTEAERIGLLKIDFLGLRNLSIIHQILTRVEKDLGFN----IDIEKIPFDDQKVFELL
E. coli     FDKSDVEYAGIVKFDFLGLRTLTIINWALEMINKRRAKNGEPPLDIAAIPLDDKKSFDML
Sal. typ    FDKSDVEYAGIVKFDFLGLRTLTIINWALEMINKRRAKNGEPPLDIAAIPLDDKKSFDML
             .      .* ** ******* *  *  *   ** .  ** .    ** ** **     *

S. aureus   SQGDTTGIFQLESDGVRSVLKKLKPEHFEDIVAVTSLYRPGPMEE--IPTYITRRHDPS-
E. coli     QRSETTAVFQLESRGMKDLIKRLQPDCFEDMIALVALFRPGPLQSGMVDNFIDRKHGREE
Sal. typ    QRSETTAVFQLESRGMKDLIKRLQPDCFEDMIALVALFRPGPLQSGMVDNFIDRKHGREE
             . . ** ******  ..* .* ** .*** .*  * .****     .    . * .

S. aureus   ----KVQYLHPHLEPILKNTYGVIIYQEQIMQIASTFANFSYGEADILRRAMSKKNRAVL
E. coli     ISYPDVQWQHESLKPVLEPTYGIILYQEQVMQIAQVLSGYTLGGADMIRRAMGKKPEEM
Sal. typ    LSYPDVQWQHESLKPVLEPTYGIILYQEQVMQIAQVLSGYTLGGADMIRRAMGKKPEEM
                 .*   *  **  **..**  ****** ***** .  .    *** **** **  .

S. aureus   ERDAQHFIEGTKQNGYHEDISKQIFDLI---------------
E. coli     AKQRSVFAEGAEKNGINAELAMKIFDLVEKFAGYGFNKSHSAAYALVSYQTIWLKAHYPA
Sal. typ    AKQRSVFEEGAKKNGIDGELAMKIFDLVEKFAGYGFNKSHSAAYALVSYQTIWLKAHYPA
             .  . * **  .** . . . * ****
```

FIGURE 9

```
                                                                    ATP site
                                                                    ‾‾‾‾‾‾‾‾
S.aureus MKGYCLWRCNLDYQALFVVPTP-KFEDVVGQEHSEDCAMG----------SHAYLFSGPRGTGKT
B.sub.   --------MSYQALYRVFRPQRFEDVVGQEHITKTLQNALLQKKFSHAYLFSGPRGTGKT
E.coli   --------MSYQVLARKWRPQTFADVVGQEHVLTALANGLSLGRIHHAYLFSGTRGVGKT
                 **   *        * ******     .                 *******  **  ***


                                        Zn++ finger
                                        ‾    ‾ ‾         ‾
S.aureus SIAKVFAKAINCLNSTDGEPCNECHICKGITQGTNSDVIEIDAASNNGVDEIRNIRDKVKYA
B.sub    SAAKIFAKAVNCEHAPVDEPCNECAACKGITNGSISDVIEIDAASNNGVDEIRDIRDKVKFA
E.coli   SIARLLAKGLNCETGITATPCGVCDNCREIEQGRFVDLIEIDAASRTKVEDTRDLLDNVQYA
         *.*  **  **        **  * *   *   * ********  .  * . . *


S.aureus SPSESKYKVYIIDEVHMLTTGAFNALLKTLEEPPAHAIFILATTEPHKIPPTIISRA
B.sub    PSAVTYKVYIIDEVHMLSIGAFNALLKTLEEPPEHCIFILATTEPHKIPLTIISRC
E.coli   PARGRFKVYLIDEVHMLSRHSFNALLKTLEEPPEHVKFLLATTDPQKLPVTILLSRC
         *    ***.*******    .******** **** .** ***.* * * ***
```

FIGURE 10

```
S.aureus   ALNIANKLERMKIYLAVGIFSLEMGADQLTTRMICSSGNVDSNRLRTGTMTEEDWSRFTI
B.sub      ALNIAQNVA-TKTDFSVAIFSLFMGAEQLVWRMLCAEGNINAQNLRTGNLTEEDWGKLTM
E.coli     AMNLVENAA-MLQDKPVLIFSLEMPSEQIMWRSLASLSRVDQTKIRTGQLDDEDWARISG
Sal.typ    AMNLCENAA-MLQDKPVLIFSLEMPGEQIMWRMLASLSRVDQTRIRTGQLDDEDWARISG
           * *    .           ***** *      .*.    .           ***  .

S.aureus   AVGKLS-RTKIFIDDTPGIPINDLRSKCRRLKQEHG-LYVIVIDYLQLIPGVGSRASDNR
B.sub      AMGSLS-NSGIYIDDIPGIRVSEIRAKCRRLKQESG-LGMILIDYLQLIQGSG-RSKDNR
E.coli     TMGILLEKRNIYIDDSSGLTPTEVRSRARRIAREHGGIGLIMIDYLQLMRVPA--LSDNR
Sal.typ    TMGILLEKRNMYIDDSSGLTPTEVRSRARRIFREHGGLSLIMIDYLQLMRVPS--LSDNR
            . *    . ***  *       .  * . * .  .   .******.         ***

S.aureus   QQEVSEISRTLKALARELECPVIADSQLSPALPPRRATRPDLPRH---------------
B.sub      QQEVSEISRELKSIARELQVPVTIALSQLSRGVEQRQDKRPMMSDIRESGSIEQDADIVAF
E.coli     TLEIAEISRSLKALAKELNVPVVALSQLNRSLEQRADKRPVNSDLRESGSIEQDADLIMF
Sal.typ    TLEIAEISRSLKALAKELQVPVVALSQLNRSLEQRADKRPVNSDLRESGSIEQDADLIMF
           .  ****  ** .* .**  ** *  ***          *               **
```

FIGURE 11

SEQUENCE LISTING

<110> The Rockefeller University

<120> DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND
      THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

<130> 22221/1003

<140>
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<150> 60/074,522
<151> 1998-01-27

<150> 60/093,727
<151> 1998-07-22

<160> 44

<170> PatentIn Ver. 2.0

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            20                  25                  30

Phe Gly His Leu Leu Ala Lys Ala Val Ala Lys Asp Val Gly Arg Ile
        35                  40                  45

Met Gly Phe Asp Glu Val Thr Leu Asn Glu Ile Ser Ser Leu Ile Pro
    50                  55                  60

His Lys Leu Gly Ile Thr Leu Asp Glu Ala Tyr Gln Ile Asp Asp Phe
65                  70                  75                  80

Lys Lys Phe Val His Arg Asn His Arg His Gln Arg Trp Phe Ser Ile
                85                  90                  95

Cys Lys Lys Leu Glu Gly Leu Pro Arg His Thr Ser Thr His Ala Ala
            100                 105                 110

Gly Ile Ile Ile Asn Asp His Pro Leu Tyr Glu Tyr Ala Pro Leu Thr
        115                 120                 125

Lys Gly Asp Thr Gly Leu Leu Thr Gln Trp Thr Met Thr Glu Ala Glu
    130                 135                 140

Arg Ile Gly Leu Leu Lys Ile Asp Phe Leu Gly Leu Arg Asn Leu Ser
145                 150                 155                 160

Ile Ile His Gln Ile Leu Thr Arg Val Glu Lys Asp Leu Gly Phe Asn
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2

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            195   200   205

Val Arg Ser Val Leu Lys Lys Leu Lys Pro Glu His Phe Glu Asp Ile
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Val Ala Val Thr Ser Leu Tyr Arg Pro Gly Pro Met Glu Glu Ile Pro
225   230   235   240

Thr Tyr Ile Thr Arg Arg His Asp Pro Ser Lys Val Gln Tyr Leu His
                245   250   255

Pro His Leu Glu Pro Ile Leu Lys Asn Thr Tyr Gly Val Ile Ile Tyr
            260   265   270

Gln Glu Gln Ile Met Gln Ile Ala Ser Thr Phe Ala Asn Phe Ser Tyr
            275   280   285

Gly Glu Ala Asp Ile Leu Arg Arg Ala Met Ser Lys Lys Asn Arg Ala
            290   295   300

Val Leu Glu Arg Asp Ala Gln His Phe Ile Glu Gly Thr Lys Gln Asn
305   310   315   320

Gly Tyr His Glu Asp Ile Ser Lys Gln Ile Phe Asp Leu Ile Leu Lys
                325   330   335

Phe Ala Asp Gly Phe Pro Arg Ala His Ala Val Ser Tyr Ser Lys Ile
            340   345   350

Ala Tyr Ile Met Ser Phe Leu Lys Val His Tyr Pro Asn Tyr Phe Tyr
            355   360   365

Ala Asn Ile Leu Ser Asn Val Ile Gly Ser Glu Lys Lys Thr Ala Gln
            370   375   380

Met Ile Glu Glu Ala Lys Lys Gln Gly Ile Thr Ile Leu Pro Pro Asn
385   390   395   400

Ile Asn Glu Ser His Trp Phe Tyr Lys Pro Ser Gln Glu Gly Ile Tyr
                405   410   415

Leu Ser Ile Gly Thr Ile Lys Gly Val Gly Tyr Gln Ser Val Lys Val
            420   425   430

3

```
Ile Val Glu Glu Arg Phe Gln Asn Gly Lys Phe Lys Asp Phe Phe Asp
        435                 440                 445

Ser Ala Arg Arg Ile Pro Lys Arg Val Lys Thr Arg Lys Leu Leu Glu
    450                 455                 460

Ala Leu Ile Leu Val Gly Ala Phe Asp Ala Phe Gly Lys Thr Arg Ser
465                 470                 475                 480

Thr Leu Leu Gln Ala Ile Asp Gln Val Leu Asp Gly Asp Leu Asn Ile
            485                 490                 495

    Glu Gln Asp Gly Phe Leu Phe Asp Ile Leu Thr Pro Lys Gln Met Tyr
            500                 505                 510

    Glu Asp Lys Glu Glu Leu Pro Asp Ala Leu Ile Ser Gln Tyr Glu Lys
            515                 520                 525

    Glu Tyr Leu Gly Phe Tyr Val Ser Gln His Pro Val Asp Lys Lys Phe
        530                 535                 540

Val Ala Lys Gln Tyr Leu Thr Ile Phe Ser Cys Glu Asn Val Ala Lys
545                 550                 555                 560

Asp Val Arg Arg Ile Met Gly Phe Asp Glu Val Lys Gln
                565                 570
```

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<210> 3
<211> 1698
<212> DNA
<213> Staphylococcus aureus

<400> 3
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gttgatgaaa taagaaatat tagagacaaa gttaaatatg caccaagtga atcgaaatat 360
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atggatcagg ctattgcttt cggcgatggc acattgacat acaagatgc cctaaatgtt 720
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```
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<210> 4
<211> 566
<212> PRT
<213> Staphylococcus aureus
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<400> 4
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 1               5                  10                  15


Asp Val Val Gly Gln Glu His Val Thr Lys Thr Leu Arg Asn Ala Ile
                20                  25                  30


Ser Lys Glu Lys Gln Ser His Ala Tyr Ile Phe Ser Gly Pro Arg Gly
            35                  40                  45


Thr Gly Lys Thr Ser Ile Ala Lys Val Phe Ala Lys Ala Ile Asn Cys
        50                  55                  60


Leu Asn Ser Thr Asp Gly Glu Pro Cys Asn Glu Cys His Ile Cys Lys
65                  70                  75                  80


Gly Ile Thr Gln Gly Thr Asn Ser Asp Val Ile Glu Ile Asp Ala Ala
                85                  90                  95


Ser Asn Asn Gly Val Asp Glu Ile Arg Asn Ile Arg Asp Lys Val Lys
            100                 105                 110


Tyr Ala Pro Ser Glu Ser Lys Tyr Lys Val Tyr Ile Ile Asp Glu Val
            115                 120                 125


His Met Leu Thr Thr Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu
```

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        130                    135                    140

Glu Pro Pro Ala His Ala Ile Phe Ile Leu Ala Thr Thr Glu Pro His
145                 150                 155                 160

Lys Ile Pro Pro Thr Ile Ile Ser Arg Ala Gln Arg Phe Asp Phe Lys
                165                 170                 175

Ala Ile Ser Leu Asp Gln Ile Val Glu Arg Leu Lys Phe Val Ala Asp
            180                 185                 190

Ala Gln Gln Ile Glu Cys Glu Asp Glu Ala Leu Ala Phe Ile Ala Lys
        195                 200                 205

Ala Ser Glu Gly Gly Met Arg Asp Ala Leu Ser Ile Met Asp Gln Ala
    210                 215                 220

Ile Ala Phe Gly Asp Gly Thr Leu Thr Leu Gln Asp Ala Leu Asn Val
225                 230                 235                 240

Thr Gly Ser Val His Asp Glu Ala Leu Asp His Leu Phe Asp Asp Ile
            245                 250                 255

Val Gln Gly Asp Val Gln Ala Ser Phe Lys Lys Tyr His Gln Phe Ile
            260                 265                 270

Thr Glu Gly Lys Glu Val Asn Arg Leu Ile Asn Asp Met Ile Tyr Phe
        275                 280                 285

Val Arg Asp Thr Ile Met Asn Lys Thr Ser Glu Lys Asp Thr Glu Tyr
    290                 295                 300

Arg Ala Leu Met Asn Leu Glu Leu Asp Met Leu Tyr Gln Met Ile Asp
305                 310                 315                 320

Leu Ile Asn Asp Thr Leu Val Ser Ile Arg Phe Ser Val Asn Gln Asn
                325                 330                 335

Val His Phe Glu Val Leu Leu Val Lys Leu Ala Glu Gln Ile Lys Gly
            340                 345                 350

Gln Pro Gln Val Ile Ala Asn Val Ala Glu Pro Ala Gln Ile Ala Ser
        355                 360                 365

Ser Pro Asn Thr Asp Val Leu Leu Gln Arg Met Glu Gln Leu Glu Gln
370                 375                 380

Glu Leu Lys Thr Leu Lys Ala Gln Gly Val Ser Val Ala Pro Thr Gln
```

6

```
      385                   390                   395                   400
```

Lys Ser Ser Lys Lys Pro Ala Arg Gly Ile Gln Lys Ser Lys Asn Ala
                 405   410   415

Phe Ser Met Gln Gln Ile Ala Lys Val Leu Asp Lys Ala Asn Lys Ala
             420   425   430

Asp Ile Lys Leu Leu Lys Asp His Trp Gln Glu Val Ile Asp His Ala
         435   440   445

Gln Asn Asn Asp Lys Lys Ser Leu Val Ser Leu Leu Gln Asn Ser Glu
     450   455   460

Pro Val Ala Ala Ser Glu Asp His Val Leu Val Lys Phe Glu Glu Glu
465   470   475   480

Ile His Cys Glu Ile Val Asn Lys Asp Asp Glu Lys Arg Ser Ser Ile
             485   490   495

Glu Ser Val Val Cys Asn Ile Val Asn Lys Asn Val Lys Val Val Gly
         500   505   510

Val Pro Ser Asp Gln Trp Gln Arg Val Arg Thr Glu Tyr Leu Gln Asn
     515   520   525

Arg Lys Asn Glu Gly Asp Asp Met Pro Lys Gln Gln Ala Gln Gln Thr
     530   535   540

Asp Ile Ala Gln Lys Ala Lys Asp Leu Phe Gly Glu Glu Thr Val His
545   550   555   560

Val Ile Asp Glu Glu Glx
             565

<210> 5
<211> 2034
<212> DNA
<213> Staphylococcus aureus

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gagtcgtttt ataggggtgc ccatcaacat attttccgtg caatgatgca cttaaatgaa 180
gataataaag aaattgatgt tgtaacattg atggatcaat tatcgacgga aggtacgttg 240
aatgaagcgg tggcccgca atatcttgca gagttatcta caaatgtacc aacgacgcga 300
aatgttcagt attatactga tatcgtttct aagcatgcat taaaacgtag attgattcaa 360
```

7

```
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gacattcgag acgtcttagg acaagtgtat gaaacagctg aagagcttga tcaaaatagt 540
ggtcaaacac caggtatacc tacaggatat cgagatttag accaaatgac agcagggttc 600
aaccgaaatg atttaattat ccttgcagcg cgtccatctg taggtaagac tgcgttcgca 660
cttaatattg cacaaaaagt tgcaacgcat gaagatatgt atacagttaa aagcaacagg 720
aagtttctga aatctctcgt acattaaaag cattagcccg tgaattaaaa tgtccagtta 780
tcgcattaag tcagttatct cgtggtgttg aacaacgaca agataaacgt ccaatgatga 840
gtgatattcg tgaatctggt tcgattgagc aagatgccga tatcgttgca ttcttatacc 900
gtgatgatta ctataaccgt ggcggcgatg aagatgatga cgatgatggt ggtttcgagc 960
cacaaacgaa tgatgaaaac ggtgaaattg aaattatcat tgttaagcaa cgtaacggtc 1020
caacaggcac agttaagtta cattttatga aacaatataa taaattttag agctatcatc 1080
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gttggtaaat tatcacgtac gaagattttt attgatgata caccgggtat tcgaattaat 1560
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gtttctgaaa tctctcgtac attaaaagca ttagcccgtg aattaaaatg tccagttatc 1740
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```

<210> 6
<211> 457
<212> PRT
<213> Staphylococcus aureus

<400> 6

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Met Asp Arg Met Tyr Glu Gln Asn Gln Met Pro His Asn Asn Glu Ala
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Glu Gln Ser Val Leu Gly Ser Ile Ile Ile Asp Pro Glu Leu Ile Asn
             20                  25                  30

Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His
         35                  40                  45

Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu
     50                  55                  60

Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu
```

8

```
           65                    70                   75                   80

     Asn Glu Ala Gly Gly Pro Gln Tyr Leu Ala Glu Leu Ser Thr Asn Val
                     85                   90                   95

     Pro Thr Thr Arg Asn Val Gln Tyr Tyr Thr Asp Ile Val Ser Lys His
                 100                  105                  110

     Ala Leu Lys Arg Arg Leu Ile Gln Thr Ala Asp Ser Ile Ala Asn Asp
             115                  120                  125

     Gly Tyr Asn Asp Glu Leu Glu Leu Asp Ala Ile Leu Ser Asp Ala Glu
             130                  135                  140

     Arg Arg Ile Leu Glu Leu Ser Ser Ser Arg Glu Ser Asp Gly Phe Lys
     145                  150                  155                  160

     Asp Ile Arg Asp Val Leu Gly Gln Val Tyr Glu Thr Ala Glu Glu Leu
                 165                  170                  175

     Asp Gln Asn Ser Gly Gln Thr Pro Gly Ile Pro Thr Gly Tyr Arg Asp
                 180                  185                  190

     Leu Asp Gln Met Thr Ala Gly Phe Asn Arg Asn Asp Leu Ile Ile Leu
                 195                  200                  205

     Ala Ala Arg Pro Ser Val Gly Lys Thr Ala Phe Ala Leu Asn Ile Ala
             210                  215                  220

     Gln Lys Leu Glu Arg Met Lys Ile Tyr Leu Ala Val Gly Ile Phe Ser
     225                  230                  235                  240

     Leu Glu Met Gly Ala Asp Gln Leu Thr Thr Arg Met Ile Cys Ser Ser
                 245                  250                  255

     Gly Asn Val Asp Ser Asn Arg Leu Arg Thr Gly Thr Met Thr Glu Glu
                 260                  265                  270

     Asp Trp Ser Arg Phe Thr Ile Ala Val Gly Lys Leu Ser Arg Thr Lys
                 275                  280                  285

     Ile Phe Ile Asp Asp Thr Pro Gly Ile Arg Ile Asn Asp Leu Arg Ser
             290                  295                  300

     Lys Cys Arg Arg Leu Lys Gln Glu His Gly Leu Asp Met Ile Val Ile
     305                  310                  315                  320

     Asp Tyr Leu Gln Leu Ile Gln Gly Ser Gly Ser Arg Ala Ser Asp Asn
```

9

```
                    325                    330                    335

    Arg Gln Gln Glu Val Ser Glu Ile Ser Arg Thr Leu Lys Ala Leu Ala
            340                    345                    350


    Arg Glu Leu Lys Cys Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly
            355                    360                    365


    Val Glu Gln Arg Gln Asp Lys Arg Pro Met Met Ser Asp Ile Arg Glu
            370                    375                    380


    Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg
    385                    390                    395                    400


    Asp Asp Tyr Tyr Asn Arg Gly Gly Asp Glu Asp Asp Asp Asp Gly
        ·             405                    410                    415


    Gly Phe Glu Pro Gln Thr Asn Asp Glu Asn Gly Glu Ile Glu Ile Ile
            420                    425                    430


    Ile Ala Lys Gln Arg Tyr Gly Pro Gly Thr Val Lys Leu Leu Phe Met
            435                    440                    445


    Lys Gln Tyr Gly Lys Phe Thr Asp Ile
        450                    455
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<210> 7
<211> 4266
<212> DNA
<213> Staphylococcus aureus

<400> 7
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acatggaat tcatattac attaccacaa ttcttagctc atgaagatta tttattattt 180
ataaatgcaa tagagcaaga gtttaaagat atcgccaacg ttacatgtcg ttttacggta 240
acaaatggca cgaatcaaga tgaacatgca attaaatact ttgggcactg tattgaccaa 300
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gaaacaaatg ataatgatca agaacaaaac ttagcttctt tagaagcaca tattcaagaa 540
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gcgaaacaac aagataacaa agcaaagtgct gtcgataagt gtcaaattgg taagccgatt 660
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attaaagtga ctgactatac ggactcttta gtttttaaaaa tgtttactcg taaaaacaaa 840
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11

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```

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<210> 8
<211> 1435
<212> PRT
<213> Staphylococcus aureus

<400> 8
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  1               5                  10                  15

Ser Asn Gln Leu Asp Ala Glu Ile Leu Asn Ser Gly Glu Leu Thr Arg
            20                  25                  30

Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
        35                  40                  45

Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
    50                  55                  60

Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
 65                  70                  75                  80

Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His
                85                  90                  95

Cys Ile Asp Gln Thr Ala Leu Ser Pro Lys Val Lys Gly Gln Leu Lys
            100                 105                 110

Gln Lys Lys Leu Ile Met Ser Gly Lys Val Leu Lys Val Met Val Ser
        115                 120                 125

Asn Asp Ile Glu Arg Asn His Phe Asp Lys Ala Cys Asn Gly Ser Leu
    130                 135                 140

Ile Lys Ala Phe Arg Asn Cys Gly Phe Asp Ile Asp Lys Ile Ile Phe
145                 150                 155                 160

Glu Thr Asn Asp Asn Asp Gln Glu Gln Asn Leu Ala Ser Leu Glu Ala
                165                 170                 175
```

```
His Ile Gln Glu Glu Asp Glu Gln Ser Ala Arg Leu Ala Thr Glu Lys
        180                 185                 190

Leu Glu Lys Met Lys Ala Glu Lys Ala Lys Gln Gln Asp Asn Lys Gln
        195                 200                 205

Ser Ala Val Asp Lys Cys Gln Ile Gly Lys Pro Ile Gln Ile Glu Asn
    210                 215                 220

Ile Lys Pro Ile Glu Ser Ile Ile Glu Glu Glu Phe Lys Val Ala Ile
225                 230                 235                 240

Glu Gly Val Ile Phe Asp Ile Asn Leu Lys Glu Leu Lys Ser Gly Arg
                245                 250                 255

His Ile Val Glu Ile Lys Val Thr Asp Tyr Thr Asp Ser Leu Val Leu
        260                 265                 270

Lys Met Phe Thr Arg Lys Asn Lys Asp Asp Leu Glu His Phe Lys Ala
        275                 280                 285

Leu Ser Val Gly Lys Trp Val Arg Ala Gln Gly Arg Ile Glu Glu Asp
        290                 295                 300

Thr Phe Ile Arg Asp Leu Val Met Met Met Ser Asp Ile Glu Glu Ile
305                 310                 315                 320

Lys Lys Ala Thr Lys Lys Asp Lys Ala Glu Glu Lys Arg Val Glu Phe
                325                 330                 335

His Leu His Thr Ala Met Ser Gln Met Asp Gly Ile Pro Asn Ile Gly
        340                 345                 350

Ala Tyr Val Lys Gln Ala Ala Asp Trp Gly His Pro Ala Ile Ala Val
        355                 360                 365

Thr Asp His Asn Val Val Gln Ala Phe Pro Asp Ala His Ala Ala Ala
    370                 375                 380

Glu Lys His Gly Ile Lys Met Ile Tyr Gly Met Glu Gly Met Leu Val
385                 390                 395                 400

Asp Asp Gly Val Pro Ile Ala Tyr Lys Pro Gln Asp Val Val Leu Lys
                405                 410                 415

Asp Ala Thr Tyr Val Val Phe Asp Val Glu Thr Thr Gly Leu Ser Asn
        420                 425                 430
```

13

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Gln Tyr Asp Lys Ile Ile Glu Leu Ala Ala Val Lys Val His Asn Gly
        435                 440                 445

Glu Ile Ile Asp Lys Phe Glu Arg Phe Ser Asn Pro His Glu Arg Leu
        450                 455                 460

Ser Glu Thr Ile Ile Asn Leu Thr His Ile Thr Asp Asp Met Leu Val
465                 470                 475                 480

Asp Ala Pro Glu Ile Glu Glu Val Leu Thr Glu Phe Lys Glu Trp Val
                485                 490                 495

Gly Asp Ala Ile Phe Val Ala His Asn Ala Ser Phe Asp Met Gly Phe
            500                 505                 510

Ile Asp Thr Gly Tyr Glu Arg Leu Gly Phe Gly Pro Ser Thr Asn Gly
        515                 520                 525

Val Ile Asp Thr Leu Glu Leu Ser Arg Thr Ile Asn Thr Glu Tyr Gly
        530                 535                 540

Lys His Gly Leu Asn Phe Leu Ala Lys Lys Tyr Gly Val Glu Leu Thr
545                 550                 555                 560

Gln His His Arg Ala Ile Tyr Asp Thr Glu Ala Thr Ala Tyr Ile Phe
                565                 570                 575

Ile Lys Met Val Gln Gln Met Lys Glu Leu Gly Val Leu Asn His Asn
            580                 585                 590

Glu Ile Asn Lys Lys Leu Ser Asn Glu Asp Ala Tyr Lys Arg Ala Arg
        595                 600                 605

Pro Ser His Val Thr Leu Ile Val Gln Asn Gln Gln Gly Leu Lys Asn
    610                 615                 620

Leu Phe Lys Ile Val Ser Ala Ser Leu Val Lys Tyr Phe Tyr Arg Thr
625                 630                 635                 640

Pro Arg Ile Pro Arg Ser Leu Leu Asp Glu Tyr Arg Glu Gly Leu Leu
                645                 650                 655

Val Gly Thr Ala Cys Asp Glu Gly Glu Leu Phe Thr Ala Val Met Gln
            660                 665                 670

Lys Asp Gln Ser Gln Val Glu Lys Ile Ala Lys Tyr Tyr Asp Phe Ile
        675                 680                 685
```

14

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Glu Ile Gln Pro Pro Ala Leu Tyr Gln Asp Leu Ile Asp Arg Glu Leu
    690             695             700

Ile Arg Asp Thr Glu Thr Leu His Glu Ile Tyr Gln Arg Leu Ile His
705             710             715                 720

Ala Gly Asp Thr Ala Gly Ile Pro Val Ile Ala Thr Gly Asn Ala His
            725             730             735

Tyr Leu Phe Glu His Asp Gly Ile Ala Arg Lys Ile Leu Ile Ala Ser
        740             745             750

Gln Pro Gly Asn Pro Leu Asn Arg Ser Thr Leu Pro Glu Ala His Phe
        755             760             765

Arg Thr Thr Asp Glu Met Leu Asn Glu Phe His Phe Leu Gly Glu Glu
    770             775             780

Lys Ala His Glu Ile Val Val Lys Asn Thr Asn Glu Leu Ala Asp Arg
785             790             795                 800

Ile Glu Arg Val Val Pro Ile Lys Asp Glu Leu Tyr Thr Pro Arg Met
            805             810             815

Glu Gly Ala Asn Glu Glu Ile Arg Glu Leu Ser Tyr Ala Asn Ala Arg
            820             825             830

Lys Leu Tyr Gly Glu Asp Leu Pro Gln Ile Val Ile Asp Arg Leu Glu
        835             840             845

Lys Glu Leu Lys Ser Ile Ile Gly Asn Gly Phe Ala Val Ile Tyr Leu
    850             855             860

Ile Ser Gln Arg Leu Val Lys Lys Ser Leu Asp Asp Gly Tyr Leu Val
865             870             875                 880

Gly Ser Arg Gly Ser Val Gly Ser Ser Phe Val Ala Thr Met Thr Glu
            885             890             895

Ile Thr Glu Val Asn Pro Leu Pro Pro His Tyr Ile Cys Pro Asn Cys
        900             905             910

Lys Thr Ser Glu Phe Phe Asn Asp Gly Ser Val Gly Ser Gly Phe Asp
        915             920             925

Leu Pro Asp Lys Thr Cys Glu Thr Cys Gly Ala Pro Leu Ile Lys Glu
    930             935             940
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15

Gly Gln Asp Ile Pro Phe Glu Lys Phe Leu Gly Phe Lys Gly Asp Lys
945   950   955   960

Val Pro Asp Ile Asp Leu Asn Phe Ser Gly Glu Tyr Gln Pro Asn Ala
                965   970   975

His Asn Tyr Thr Lys Val Leu Phe Gly Glu Asp Lys Val Phe Arg Ala
                980   985   990

Gly Thr Ile Gly Thr Val Ala Glu Lys Thr Ala Phe Gly Tyr Val Lys
            995   1000   1005

Gly Tyr Leu Asn Asp Gln Gly Ile His Lys Arg Gly Ala Glu Ile Asp
    1010   1015   1020

Arg Leu Val Lys Gly Cys Thr Gly Val Arg Ala Thr Thr Gly Gln His
1025   1030   1035   1040

Pro Gly Gly Ile Ile Val Val Pro Asp Tyr Met Asp Ile Tyr Asp Phe
            1045   1050   1055

Thr Pro Ile Gln Tyr Pro Ala Asp Asp Gln Asn Ser Ala Trp Met Thr
            1060   1065   1070

Thr His Phe Asp Phe His Ser Ile His Asp Asn Val Leu Lys Leu Asp
            1075   1080   1085

Ile Leu Gly His Asp Asp Pro Thr Met Ile Arg Met Leu Gln Asp Leu
    1090   1095   1100

Ser Gly Ile Asp Pro Lys Thr Ile Pro Val Asp Asp Lys Glu Val Met
1105   1110   1115   1120

Gln Ile Phe Ser Thr Pro Glu Ser Leu Gly Val Thr Glu Asp Glu Ile
            1125   1130   1135

Leu Cys Lys Thr Gly Thr Phe Gly Val Pro Asn Ser Asp Arg Ile Arg
            1140   1145   1150

Arg Gln Met Leu Glu Asp Thr Lys Pro Thr Thr Phe Ser Glu Leu Val
    1155   1160   1165

Gln Ile Ser Gly Leu Ser His Gly Thr Asp Val Trp Leu Gly Asn Ala
    1170   1175   1180

Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly
1185   1190   1195   1200

16

```
Cys Arg Asp Asp Ile Met Val Tyr Leu Met Tyr Ala Gly Leu Glu Pro
          1205                    1210                    1215

Ser Met Ala Phe Lys Ile Met Glu Ser Val Arg Lys Gly Lys Gly Leu
          1220                    1225                    1230

Thr Glu Glu Met Ile Glu Thr Met Lys Glu Asn Glu Val Pro Asp Trp
          1235                    1240                    1245

Tyr Leu Asp Ser Cys Leu Lys Ile Lys Tyr Ile Phe Pro Lys Ala His
          1250                    1255                    1260

Ala Ala Ala Tyr Val Leu Met Ala Val Arg Ile Ala Tyr Phe Lys Val
1265                1270                1275                1280

His His Pro Leu Tyr Tyr Tyr Ala Ser Tyr Phe Thr Ile Arg Ala Ser
                1285              .     1290                1295

Asp Phe Asp Leu Ile Thr Met Ile Lys Asp Lys Thr Ser Ile Arg Asn
          1300                    1305                    1310

Thr Val Lys Asp Met Tyr Ser Arg Tyr Met Asp Leu Gly Lys Lys Glu
          1315                    1320                    1325

Lys Asp Val Leu Thr Val Leu Glu Ile Met Asn Glu Met Ala His Arg
          1330                    1335                    1340

Gly Tyr Arg Met Gln Pro Ile Ser Leu Glu Lys Ser Gln Ala Phe Glu
1345                1350                1355                1360

Phe Ile Ile Glu Gly Asp Thr Leu Ile Pro Pro Phe Ile Ser Val Pro
                1365                    1370                    1375

Gly Leu Gly Glu Asn Val Ala Lys Arg Ile Val Glu Ala Arg Asp Asp
          1380              .     1385                1390

Gly Pro Phe Leu Ser Lys Glu Asp Leu Asn Lys Lys Ala Gly Leu Tyr
          1395                    1400                    1405

Gln Lys Ile Ile Glu Tyr Leu Asp Glu Leu Gly Ser Leu Pro Asn Leu
          1410                    1415                    1420

Pro Asp Lys Ala Gln Leu Ser Ile Phe Asp Met
1425                1430                1435
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`<210> 9`

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<211> 1134
<212> DNA
<213> Staphylococcus aureus

<400> 9
atgatggaat tcactattaa aagagattat tttattacac aattaaatga cacattaaaa  60
gctatttcac caagaacaac attacctata ttaactggta tcaaaatcga tgcgaaagaa 120
catgaagtta tattaactgg ttcagactct gaaatttcaa tagaaatcac tattcctaaa 180
actgtagatg gcgaagatat tgtcaatatt tcagaaacag gctcagtagt acttcctgga 240
cgattctttg ttgatattat aaaaaaatta cctggtaaag atgttaaatt atctacaaat 300
gaacaattcc agacattaat tacatcaggt cattctgaat ttaatttgag tggcttagat 360
ccagatcaat atcctttatt acctcaagtt tctagagatg acgcaattca attgtcggta 420
aaagtactta aaaacgtgat tgcacaaacg aattttgcag tgtccacctc agaaacacgc 480
ccagtactaa ctggtgtgaa ctggcttata caagaaaatg aattaatatg cacagcgact 540
gattcacacc gcttggctgt aagaaagttg cagttagaag atgtttctga aacaaaaat  600
gtcatcattc caggtaaggc tttagctgaa ttaaataaaa ttatgtctga caatgaagaa 660
gacattgata tcttctttgc ttcaaaccaa gttttatta aagttggaaa tgtgaacttt  720
atttctcgat tattagaagg acattatcct gatacaacac gtttattccc tgaaaactat 780
gaaattaaat taagtataga caatggggag ttttatcatg cgattgatcg tgcctcttta 840
ttagcacgtg aaggtggtaa taacgttatt aaattaagta caggtgatga cgttgttgaa 900
ttatcttcta catcaccaga aattggtact gtaaaagaag aagttgatgc aaacgatgtt 960
gaaggtggta gcctgaaaat ttcattcaac tctaaatata tgatggatgc tttaaaagca 1020
atcgataatg atgaggttga agttgaattc ttcggtacaa tgaaaccatt tattctaaaa 1080
ccaaaaggtg acgactcggt aacgcaatta attttaccaa tcagaactta ctaa        1134
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<210> 10
<211> 377
<212> PRT
<213> Staphylococcus aureus

<400> 10
Met Met Glu Phe Thr Ile Lys Arg Asp Tyr Phe Ile Thr Gln Leu Asn
 1               5                  10                  15

Asp Thr Leu Lys Ala Ile Ser Pro Arg Thr Thr Leu Pro Ile Leu Thr
            20                  25                  30

Gly Ile Lys Ile Asp Ala Lys Glu His Glu Val Ile Leu Thr Gly Ser
            35                  40                  45

Asp Ser Glu Ile Ser Ile Glu Ile Thr Ile Pro Lys Thr Val Asp Gly
        50                  55                  60

Glu Asp Ile Val Asn Ile Ser Glu Thr Gly Ser Val Val Leu Pro Gly
65                  70                  75                  80

Arg Phe Phe Val Asp Ile Ile Lys Lys Leu Pro Gly Lys Asp Val Lys
                85                  90                  95
```

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Leu Ser Thr Asn Glu Gln Phe Gln Thr Leu Ile Thr Ser Gly His Ser
            100                 105                 110

Glu Phe Asn Leu Ser Gly Leu Asp Pro Asp Gln Tyr Pro Leu Leu Pro
        115                 120                 125

Gln Val Ser Arg Asp Asp Ala Ile Gln Leu Ser Val Lys Val Leu Lys
    130                 135                 140

Asn Val Ile Ala Gln Thr Asn Phe Ala Val Ser Thr Ser Glu Thr Arg
145                 150                 155                 160

Pro Val Leu Thr Gly Val Asn Trp Leu Ile Gln Glu Asn Glu Leu Ile
                165                 170                 175

Cys Thr Ala Thr Asp Ser His Arg Leu Ala Val Arg Lys Leu Gln Leu
            180                 185                 190

Glu Asp Val Ser Glu Asn Lys Asn Val Ile Ile Pro Gly Lys Ala Leu
        195                 200                 205

Ala Glu Leu Asn Lys Ile Met Ser Asp Asn Glu Glu Asp Ile Asp Ile
    210                 215                 220

Phe Phe Ala Ser Asn Gln Val Leu Phe Lys Val Gly Asn Val Asn Phe
225                 230                 235                 240

Ile Ser Arg Leu Leu Glu Gly His Tyr Pro Asp Thr Thr Arg Leu Phe
                245                 250                 255

Pro Glu Asn Tyr Glu Ile Lys Leu Ser Ile Asp Asn Gly Glu Phe Tyr
                260                 265                 270

His Ala Ile Asp Arg Ala Ser Leu Leu Ala Arg Glu Gly Gly Asn Asn
            275                 280                 285

Val Ile Lys Leu Ser Thr Gly Asp Asp Val Val Glu Leu Ser Ser Thr
    290                 295                 300

Ser Pro Glu Ile Gly Thr Val Lys Glu Glu Val Asp Ala Asn Asp Val
305                 310                 315                 320

Glu Gly Gly Ser Leu Lys Ile Ser Phe Asn Ser Lys Tyr Met Met Asp
                325                 330                 335

Ala Leu Lys Ala Ile Asp Asn Asp Glu Val Glu Val Glu Phe Phe Gly
            340                 345                 350
```

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Thr Met Lys Pro Phe Ile Leu Lys Pro Lys Gly Asp Asp Ser Val Thr
        355                 360                 365

Gln Leu Ile Leu Pro Ile Arg Thr Tyr
    370                 375
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<210> 11
<211> 1719
<212> DNA
<213> Staphylococcus aureus

<400> 11
atgataggtt tgtgtccttt tcatgatgaa aagacacctt catttacagt ttctgaagat 60
aaacaaatct gtcattgttt tggttgtaaa aaaggtggca atgtttttca atttactcaa 120
gaaattaaag acatatcatt tgttgaagcg gttaaagaat taggtgatag agttaatgtt 180
gctgtagata ttgaggcaac acaatctaac tcaaatgttc aaattgcttc tgatgattta 240
caaatgattg aaatgcatga gttaatacaa gaattttatt attacgcttt aacaaagaca 300
gtcgaaggcg aacaagcatt aacatactta caagaacgtg ttttacagat gcgcttatt 360
aaagagcgag gcattggctt tgcacccgat agctcacatt tttgtcatga ttttcttcaa 420
aaaaagggtt acgatattga attagcatat gaagccggat tattatcacg taacgaagaa 480
aatttcagtt attacgatag atttcgaaat cgtattatgt ttcctttgaa aaatgcgcaa 540
ggaagaattg ttggatattc aggtcgaaca tataccggtc aagaaccaaa atacctaaat 600
agtcctgaaa cgcctatctt tcaaaaaaga aagttgttat ataacttaga taaagcacgt 660
aaatcaatta gaaaattaga tgaaattgta ttactagaag gttttatgga tgttataaaa 720
tctgatactg ctggcttgaa aaacgttgtt gcaacaatgg gtacacagtt gtcagatgaa 780
catattacct ttatacgaaa gttaacatca aatataacat taatgtttga tggggatttt 840
gcgggtagtg aagcaacact aaaacaggt caacatttgt tacagcaagg ctaaatgta 900
tttgttatac aattgccatc tggcatggat ccggatgaat acattggtaa gtatggcaac 960
gacgcattta ctactttgt aaaaaatgac aaaaagtcat ttgcacatta taagtaagt 1020
atattaaaag atgaaattgc acataatgac ctttcatatg aacgttattt gaaagaactg 1080
agtcatgaca tttcacttat gaagtcatca attctgcaac aaaaggctat aaatgatgtt 1140
gcgccatttt tcaatgttag tcctgagcag ttagctaacg aaatacaatt caatcaagca 1200
ccagccaatt attatccaga agatgagtat ggcggttatg atgagtatgg cggttatatt 1260
gaacctgagc caattggtat ggcacaattt gacaatttga gccgtcgaga aaaagcggag 1320
cgagcatttt taaaacattt aatgagagat aaagatacat ttttaaatta ttatgaaagt 1380
gttgataagg ataacttcac aaatcagcat tttaaatatg tattcgaagt cttacatgat 1440
ttttatgcgg aaaatgatca atataaatatc agtgatgctg tgcagtatgt taattcaaat 1500
gagttgagag aaacactaat tagcttagaa caatataatt tgaatggcga accatatgaa 1560
aatgaaattg atgattatgt caatgttatt aatgaaaaag acaagaaac aattgagtca 1620
ttgaatcata aattaaggga agctacaagg attggcgatg tagaattaca aaaatactat 1680
ttacagcaaa ttgttgctaa gaataaagaa cgcatgtag 1719
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<210> 12
<211> 572
<212> PRT
<213> Staphylococcus aureus
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20

<400> 12

Met Ile Gly Leu Cys Pro Phe His Asp Glu Lys Thr Pro Ser Phe Thr
 1   5   10   15

Val Ser Glu Asp Lys Gln Ile Cys His Cys Phe Gly Cys Lys Lys Gly
            20   25   30

Gly Asn Val Phe Gln Phe Thr Gln Glu Ile Lys Asp Ile Ser Phe Val
        35   40   45

Glu Ala Val Lys Glu Leu Gly Asp Arg Val Asn Val Ala Val Asp Ile
    50   55   60

Glu Ala Thr Gln Ser Asn Ser Asn Val Gln Ile Ala Ser Asp Asp Leu
65   70   75   80

Gln Met Ile Glu Met His Glu Leu Ile Gln Glu Phe Tyr Tyr Tyr Ala
                85   90   95

Leu Thr Lys Thr Val Glu Gly Glu Gln Ala Leu Thr Tyr Leu Gln Glu
            100   105   110

Arg Gly Phe Thr Asp Ala Leu Ile Lys Glu Arg Gly Ile Gly Phe Ala
        115   120   125

Pro Asp Ser Ser His Phe Cys His Asp Phe Leu Gln Lys Lys Gly Tyr
    130   135   140

Asp Ile Glu Leu Ala Tyr Glu Ala Gly Leu Leu Ser Arg Asn Glu Glu
145   150   155   160

Asn Phe Ser Tyr Tyr Asp Arg Phe Arg Asn Arg Ile Met Phe Pro Leu
                165   170   175

Lys Asn Ala Gln Gly Arg Ile Val Gly Tyr Ser Gly Arg Thr Tyr Thr
            180   185   190

Gly Gln Glu Pro Lys Tyr Leu Asn Ser Pro Glu Thr Pro Ile Phe Gln
            195   200   205

Lys Arg Lys Leu Leu Tyr Asn Leu Asp Lys Ala Arg Lys Ser Ile Arg
    210   215   220

Lys Leu Asp Glu Ile Val Leu Leu Glu Gly Phe Met Asp Val Ile Lys
225   230   235   240

Ser Asp Thr Ala Gly Leu Lys Asn Val Val Ala Thr Met Gly Thr Gln

21

                    245   250   255

   Leu Ser Asp Glu His Ile Thr Phe Ile Arg Lys Leu Thr Ser Asn Ile
               260   265   270

   Thr Leu Met Phe Asp Gly Asp Phe Ala Gly Ser Glu Ala Thr Leu Lys
               275   280   285

   Thr Gly Gln His Leu Leu Gln Gln Gly Leu Asn Val Phe Val Ile Gln
               290   295   300

   Leu Pro Ser Gly Met Asp Pro Asp Glu Tyr Ile Gly Lys Tyr Gly Asn
   305   310   315   320

   Asp Ala Phe Thr Thr Phe Val Lys Asn Asp Lys Lys Ser Phe Ala His
                        325   330   335

   Tyr Lys Val Ser Ile Leu Lys Asp Glu Ile Ala His Asn Asp Leu Ser
               340   345   350

   Tyr Glu Arg Tyr Leu Lys Glu Leu Ser His Asp Ile Ser Leu Met Lys
               355   360   365

   Ser Ser Ile Leu Gln Gln Lys Ala Ile Asn Asp Val Ala Pro Phe Phe
   370   375   380

   Asn Val Ser Pro Glu Gln Leu Ala Asn Glu Ile Gln Phe Asn Gln Ala
   385   390   395   400

   Pro Ala Asn Tyr Tyr Pro Glu Asp Glu Tyr Gly Gly Tyr Asp Glu Tyr
                        405   410   415

   Gly Gly Tyr Ile Glu Pro Glu Pro Ile Gly Met Ala Gln Phe Asp Asn
               420   425   430

   Leu Ser Arg Arg Glu Lys Ala Glu Arg Ala Phe Leu Lys His Leu Met
               435   440   445

   Arg Asp Lys Asp Thr Phe Leu Asn Tyr Tyr Glu Ser Val Asp Lys Asp
               450   455   460

   Asn Phe Thr Asn Gln His Phe Lys Tyr Val Phe Glu Val Leu His Asp
   465   470   475   480

   Phe Tyr Ala Glu Asn Asp Gln Tyr Asn Ile Ser Asp Ala Val Gln Tyr
                        485   490   495

   Val Asn Ser Asn Glu Leu Arg Glu Thr Leu Ile Ser Leu Glu Gln Tyr

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            500                     505                     510
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Asn Leu Asn Gly Glu Pro Tyr Glu Asn Glu Ile Asp Asp Tyr Val Asn
        515   520   525

Val Ile Asn Glu Lys Gly Gln Glu Thr Ile Glu Ser Leu Asn His Lys
    530   535   540

Leu Arg Glu Ala Thr Arg Ile Gly Asp Val Glu Leu Gln Lys Tyr Tyr
545   550   555   560

Leu Gln Gln Ile Val Ala Lys Asn Lys Glu Arg Met
                565   570

<210> 13
<211> 29
<212> DNA
<213> Staphylococcus aureus

<400> 13
ggtggtaatt gtcttgcata tgacagagc   29

<210> 14
<211> 30
<212> DNA
<213> Staphylococcus aureus

<400> 14
agcgattaag tggattgccg ggttgtgatg   30

<210> 15
<211> 31
<212> DNA
<213> Staphylococcus aureus

<400> 15
agcatcacaa cccggcaatc cacttaatcg c   31

<210> 16
<211> 29
<212> DNA
<213> Staphylococcus aureus

<400> 16
gactacgcca tgggcattaa ataaatacc   29

<210> 17

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<211> 31
<212> DNA
<213> Staphylococcus aureus

<400> 17
gaagatgcat ataaacgtgc aagacctagt c   31

<210> 18
<211> 34
<212> DNA
<213> Staphylococcus aureus

<400> 18
gtctgacgca cgaattgtaa agtaagatgc atag   34

<210> 19
<211> 36
<212> DNA
<213> Staphylococcus aureus

<400> 19
cgactggaag gagttttaac atatgatgga attcac   36

<210> 20
<211> 30
<212> DNA
<213> Staphylococcus aureus

<400> 20
ttatatggat ccttagtaag ttctgattgg   30

<210> 21
<211> 15
<212> PRT
<213> Staphylococcus aureus

<400> 21
Leu Leu Phe Glu Arg Phe Leu Asn Pro Glu Arg Val Ser Met Pro
 1   5   10   15

<210> 22
<211> 15
<212> PRT
<213> Staphylococcus aureus

<400> 22
Lys Phe Ala Gly Tyr Gly Phe Asn Lys Ser His Ser Ala Ala Tyr

<210> 23
<211> 44
<212> DNA
<213> Staphylococcus aureus

<400> 23
cttctttttg aaagatttct aaataaagaa cgttattcaa tgcc   44

<210> 24
<211> 45
<212> DNA
<213> Staphylococcus aureus

<400> 24
ataagctgca gcatgacttt tattaaaacc ataacctgca aattt   45

<210> 25
<211> 39
<212> DNA
<213> Staphylococcus aureus

<400> 25
agttaaaaat gccatatttt gacgtgtttt agttctaat   39

<210> 26
<211> 42
<212> DNA
<213> Staphylococcus aureus

<400> 26
cttgcaaaag cggttgctaa agatgttgga cgaattatgg gg   42

<210> 27
<211> 10
<212> PRT
<213> Staphylococcus aureus

<400> 27
His Ala Tyr Leu Phe Ser Gly Pro Arg Gly
  1   5   10

<210> 28
<211> 11
<212> PRT

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<213> Staphylococcus aureus

<400> 28
Ala Leu Leu Lys Thr Leu Glu Glu Pro Pro Glu
  1               5                   10


<210> 29
<211> 38
<212> DNA
<213> Staphylococcus aureus

<400> 29
cgcggatccc atgcatattt attttcaggt ccaagagg                             38

<210> 30
<211> 10
<212> PRT
<213> Staphylococcus aureus

<400> 30
His Ala Tyr Leu Phe Ser Gly Pro Arg Gly
  1               5                   10


<210> 31
<211> 39
<212> DNA
<213> Staphylococcus aureus

<400> 31
ccggaattct ggtggttctt ctaatgtttt taataatgc                            39

<210> 32
<211> 11
<212> PRT
<213> Staphylococcus aureus

<400> 32
Ala Leu Leu Lys Thr Leu Glu Glu Pro Pro Glu
  1               5                   10


<210> 33
<211> 38
<212> DNA
<213> Staphylococcus aureus
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26

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<400> 33
tttgtaaagg cattacgcag gggactaatt cagatgtg                              38


<210> 34
<211> 33
<212> DNA
<213> Staphylococcus aureus


<400> 34
tatgacattc attacaaggt tctccatcag tgc                                  33


<210> 35
<211> 29
<212> DNA
<213> Staphylococcus aureus


<400> 35
gagcactgat gaacttagaa ttagatatg                                       29


<210> 36
<211> 32
<212> DNA
<213> Staphylococcus aureus


<400> 36
gatactcagt atctttctca gatgttttat tc                                   32


<210> 37
<211> 14
<212> PRT
<213> Staphylococcus aureus


<400> 37
Asp Leu Ile Ile Val Ala Ala Arg Pro Ser Met Gly Lys Thr
  1               5                 10


<210> 38
<211> 15
<212> PRT
<213> Staphylococcus aureus


<400> 38
Glu Ile Ile Ile Gly Lys Gln Arg Asn Gly Pro Ile Gly Thr Val
  1               5                 10                  15


<210> 39
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27

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<211> 41
<212> DNA
<213> Staphylococcus aureus

<400> 39
gaccttataa ttgtagctgc acgtccttct atgggaaaaa c                    41

<210> 40
<211> 48
<212> DNA
<213> Staphylococcus aureus

<400> 40
aacattatta agtcagcatc ttgttctatt gatccagatt caacgaag            48

<210> 41
<211> 45
<212> DNA
<213> Staphylococcus aureus

<400> 41
gatttgtagt tctggtaatg ttgactcaaa ccgcttaaga accgg               45

<210> 42
<211> 48
<212> DNA
<213> Staphylococcus aureus

<400> 42
atacgtgtgg ttaactgatc agcaacccat ctctagtgag aaaatacc            48

<210> 43
<211> 31
<212> DNA
<213> Staphylococcus aureus

<400> 43
cgttttaatg catgcttaga aacgatatca g                              31

<210> 44
<211> 31
<212> DNA
<213> Staphylococcus aureus

<400> 44
cattgctaag caacgttacg gtccaacagg c                              31
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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER	

IPC(6) :C07H 21/04, C12N 15/11, 15/63, 1/20, 9/00
US CL : 536/23.1, 435/320.1, 435/252.1+, 435 /183+
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

 U.S. :   536/23.1, 435/320.1, 435/252.1+, 435 /183+

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	KUNST et al. The comlete genome sequence of the Gram-positive bacterium Bacillus subtilis. Nature. 20 November 1997, Vol. 390, pages 249-256, see entire article.	1 ---- 2-3, 6-9
X - Y	SAUER et al. Sporulation and Primary Sigma Factor Homologous Genes in clostridium acetobutylicum. J Bacterol. November 1994, Vol. 176, No. 21, pages 6572-6582, see entire article.	1 ---- 2-3, 6-9
Y	FRASER et al. The Minimal Gene Complement of Mycoplasma genitalium. Science. 20 October 1995, Vol . 270, pages 397-403, see entire document.	1-3, 6-9
X	US 5,151,350 A (COLBERT et al) 29 September 1992 (29/09/92), examples 1-7.	1-9

☒ Further documents are listed in the continuation of Box C.		☐ See patent family annex.	

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
		"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 MAY 1999	**20 MAY 1999**

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Lee, Li
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/01547

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,583,026 A (O'DONNELL) 10 December 1996 (10/12/96), Summary of the Invention.	1-5

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

      Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-9

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-9, drawn to DNA molecule corresponding to dnaE and expression system.
Group II, claims 10-13, drawn to dnaE protein.
Group III, claims 14-22, drawn to DNA molecule corresponding to dnaX and expression system.
Group IV, claims 23-26, drawn to dnaX protein.
Group V, claims 27-35, drawn to DNA molecule corresponding to dnaB and expression system.
Group VI, claims 36-39, drawn to dnaB protein.
Group VII, claims 40, 50-53, 59-60, drawn to method of identifying compounds which inhibit activity of a DNA polymerase per se.
Group VIII, claims 41- 42, 59-60, drawn to method of identifying compounds which inhibit the ability of a beta subunit to stimulate a DNA polymerase.
Group IX, claims 43- 44, 59-60, drawn to method of identifying compounds which inhibit the ability of a beta subunit and a DNA polymerase to interact physically.
Group X, claims 45, 59-60, drawn to method of identifying compounds which inhibit the ability of a subunit to assemble a beta subunit on a DNA molecule.
Group XI, claims 46 - 47, 59-60, drawn to method of identifying compounds which inhibit the ability of a subunit to disassemble a beta subunit from a DNA molecule.
Group XII, claims 48- 49, 59-60, drawn to method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex.
Group XIII, claims 54, 59-60, drawn to method of identifying compounds which inhibit a DnaB helicase.
Group XIV, claims 55, 59-60, drawn to method of identifying compounds which inhibit nucleoside triphosphate activity of a DnaB helicase.
Group XV, claims 56, 59-60, drawn to method of identifying compounds which inhibit primase activity.
Group XVI, claims 57, 59-60, drawn to method of identifying compounds which inhibit the ability of a primase and a DnaB protein to interact.
Group XVII, claims 58, 59-60, drawn to method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein to interact.

The inventions listed as Groups I-XVII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I invention of a nucleotide sequence of dnaE. The special technical feature of the Group I invention of the nucleotide sequence is not present in the Group II invention of the particular isolated protein dnaE from a Gram positive bacterium and the protein of SEQ. ID. No. 2.
The special technical feature of the Group III invention of a nucleotide sequence of dnaX. The special technical feature of the Group III invention of the nucleotide sequence is not present in the Group IV invention of the particular isolated protein dnaX from a Gram positive bacterium and the protein of SEQ. ID. No. 4.
The special technical feature of the Group V invention of a nucleotide sequence of dnaB. The special technical feature of the Group V invention of the nucleotide sequence is not present in the Group VI invention of the particular isolated protein dnaB from a Gram positive bacterium and the protein of SEQ. ID. No. 6.
Group I, III and V have distinct nucleotide sequences (e.g., SEQ. ID. No.1, SEQ. ID. No.3, and SEQ. ID. No.5) encode different proteins which have diffrenet structures and biological properties while Group II, IV and VI have distinct amino acid sequences (e.g., ID. No.2, SEQ. ID. No.4, and SEQ. ID. No.6)which are different proteins having different structure and biological properties. Thus, the inventions Groups (I, III, V) and Groups (II, IV, VI,) have distinct technical features from each other and the distinct technical features are not present in each other's inventions.
Groups VII and (VIII - XVII) have distinct special technical features of particular methods from each other and the distinct special technical features particular methods are not present in each other's inventions. Those particular methods of identifying compounds have different method objective, different method steps and different reagents used. For example, the special technical feature in Group VII is particular method of identifying compounds which inhibit activity of a DNA polymerase per se, the special technical feature in Group VIII is particular method of identifying compounds which inhibit the ability of a beta subunit to stimulate a DNA polymerase, the special technical feature in Group IX is particular method of identifying compounds which inhibit the ability of a beta subunit and a DNA

polymerase to interact physically, the special technical feature in Group X is particular method of identifying compounds which inhibit the ability of a subunit to assemble a beta subunit on a DNA molecule, the special technical feature in Group XI is particular method of identifying compounds which inhibit the ability of a subunit to disassemble a beta subunit from a DNA molecule, the special technical feature in Group XII is particular method of identifying compounds which inhibit dATP/ATP binding activity of a gamma complex, the special technical feature in Group XIII is particular method of identifying compounds which inhibit a DnaB helicase, the special technical feature in Group XIV is particular method of identifying compounds which inhibit nucleoside triphosphate activity of a DnaB helicase, the special technical feature in Group XV is particular method of identifying compounds which inhibit primase activity, the special technical feature in Group XVI is particular method of identifying compounds which inhibit the ability of a primase and a DnaB protein to interact, and the special technical feature in Group XVII is particular method of identifying compounds which inhibit the ability of a DNA molecule and a DnaB protein to interact.

Since the special technical features of particular nucleotide sequences and nucleotide hybridizing (e.g., SEQ. ID. No.1, SEQ. ID. No.3, and SEQ. ID. No.5) and protein sequences (e.g., SEQ. ID. No.2, SEQ. ID. No.4, SEQ. ID. No.6) in inventions of Groups I-VI are not required in inventions of Groups VII-XVII of methods of identifying compounds, Groups I-VI and VII-XVII lack unity with each other.